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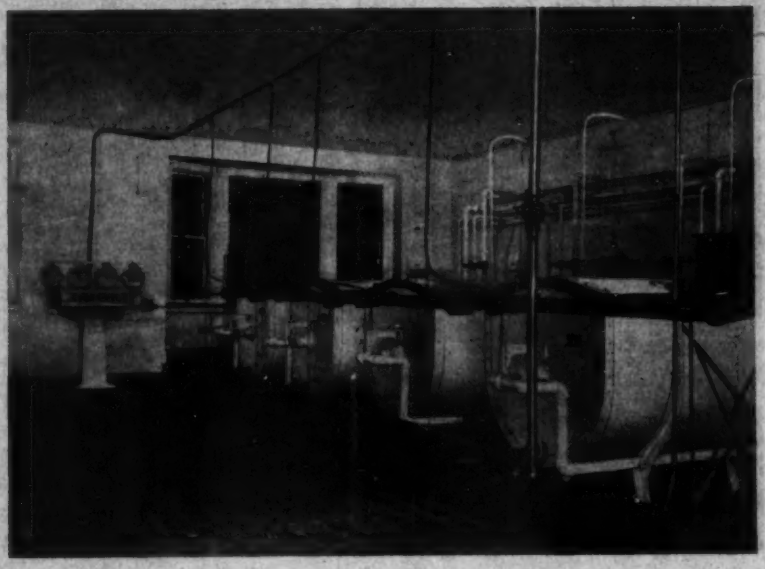

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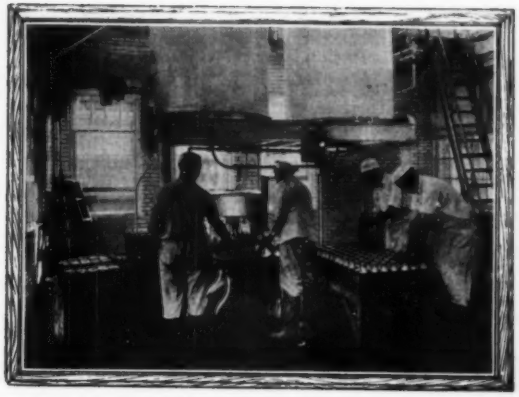

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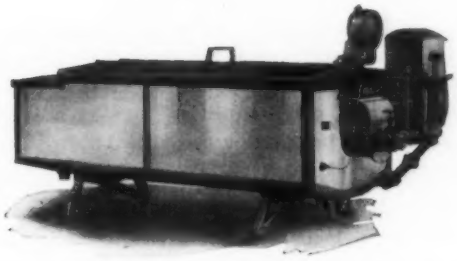

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THE EFFECT OF FAT IN THE RATION UPON THE PERCENTAGE FAT CONTENT OF THE MILK*

W. B. NEVENS, M. B. ALLEMAN AND L. T. PECK[1]

Department of Dairy Husbandry, University of Illinois, Urbana, Illinois

An attempt to increase the percentage butterfat content of cow's milk has been the object of a large number of experiments. This subject engaged the efforts of several investigators during the early years of the agricultural experiment stations in this country, and has received no little attention also on the part of research workers in Germany, The British Isles, France, Denmark and India. An adequate review of the extensive literature on this subject is precluded by lack of space.

Within recent years, the subject has attracted attention in connection with the making of high production records under the supervision of the Advanced Registry Departments of the dairy cattle breed associations.

The close supervision given these records is intended to assure the accuracy of the record and the prevention of fraud through changes of the feed or otherwise, during the period of actual inspection by the supervisor or just prior to this period. A number of contributions to the literature on this subject have appeared in this journal (1).

There are several ways in which the percentage of fat in the milk may be materially influenced; e.g., (*a*) by having the cows excessively fat at the time of calving (2). Both percentage

* Received for publication April 15, 1926.

[1] The experiments reported herewith as Series III and IV constitute a part of a research problem carried out by M. B. Alleman and L. T. Peck, graduate students in Dairy Husbandry, under the direction of W. B. Nevens. Messrs. Alleman and Peck supervised the feeding of the cows and made the butterfat tests of the milk during the experiments reported in Series III. Mr. Alleman conducted the feeding during Series IV, the butterfat tests in this case being made by laboratory assistants. Series I and II were carried on by barn and laboratory helpers under the direction of W. B. Nevens.

fat content and yield of butterfat are increased above normal. This is considered a legitimate practice and is commonly used. (*b*) By physiological underfeeding, that is shortly after calving, reducing the feed sharply below the requirements (3). This practice, however, usually reduces milk and may reduce the total fat production and is, therefore, little favored. (*c*) By feeding excessive amounts of certain high protein concentrates, such as cottonseed meal, these being added to the usual ration or substituted for part of it (4), (5). The effects on the composition of the milk induced by such changes in the feed are evidently inconstant and vary greatly with different individuals. Armsby maintains that "both experiments with pure proteins and those in which an increase in the protein content of the rations has been secured by the use of protein-rich feeds, seem to indicate clearly a stimulating influence of excess protein on milk production, although in a majority of cases the effect was not very large." (*d*) By increasing the fat content of the ration by the addition or substitution of fat or feeds high in fat. The literature dealing with investigations of this sort is extensive. One of the more recent experiments was reported by Woodward (5) in this journal.

In Woodward's experiments, high protein concentrates were substituted for one-fourth or three-fourths of the "ordinary grain," thus presumably greatly increasing the daily intake of protein. Substitution of the smaller amounts was without apparent influence on the test of the milk, but when linseed oil meal and cottonseed meal were substituted for three-fourths of the "ordinary grain" there was in most cases, an effect upon the test for a short time at least. Gluten feed seemed to be without effect. Calculation of the fat yields by means of data on milk yields, kindly supplied to the authors by Mr. Woodward, shows that substituting cottonseed meal for three-fourths of the grain in one period caused an increase of 15 per cent in fat yield, while in the case of linseed oil meal used in the same way, there were increases of from 1 to 10 per cent and decreases of the same magnitude. Usually the substitution of linseed oil meal for three-fourths of the grain mixture caused an increase in the

test of the milk, but in at least two such cases such a change depressed the fat yields 8 and 10 per cent, respectively. The addition of linseed oil to the "ordinary grain" of three cows caused an increase in the test of the milk of about 9 per cent and a decreased yield of fat of more than 5 per cent.

While the percentage of fat in the milk of a cow during a two-day test has more weight than the yield of fat, since the average percentage of fat for the two days is used as the average percentage for the month, a decreased fat yield caused by a depressed milk flow is an undesirable accompaniment. A marked lowering of the milk yield below that of the days preceding or following the test period at once raises a question regarding the correctness of the milk weights as reported by the owner for the balance of the month. In such a case also, changes in the feed or "jockeying" of the cow may be suspected.

In executing the experiments reported herewith, efforts were made to maintain the levels of protein intake at as nearly the exact requirements of the individual animals as possible, and to prevent decreases in the daily milk yields even when extreme changes in the rations were made.

OBJECT OF INVESTIGATION

The primary object of the investigation reported herewith was to study the effect of rations high in oil content upon the composition of the milk produced, especial attention being focused upon the percentage butterfat content. A secondary object in the later experiments, was to establish, if possible, the factor responsible for the changes in the percentage butterfat content of the milk which were noted in the second series of experiments.

GENERAL PLAN OF THE INVESTIGATION

The general plan of feeding the experimental animals (cows) was to feed rations high in oil content and low in oil content during alternate periods, samples of milk for analysis being taken at each milking. In Series II, a concentrate mixture

moderately high in fat content which was used in feeding the general herd constituted the check or control rations, while in the other series, a concentrate mixture formulated as being especially low in fat content was used as a control. The amounts of the feeds supplied were as closely as possible in accord with the estimated requirements of the animals. The experimental periods of Series I and II varied in length from two days to five weeks, while in Series III and IV they were seven days in length.

In Series II determinations of the percentage of fat in the milk were made by the Roese-Gottlieb method, comparison being made with the Babcock method. Determinations of total solids were made in accordance with the methods of the Association of Official Agricultural Chemists. In the other series, the Babcock method for determinations of butterfat was used. Mercury bichloride tablets were used as a preservative agent in Series I, in which butterfat tests were made weekly, while in the other series formalin was the preservative agent used whenever it was necessary. In Series III and IV, a preservative was seldom used because tests were made of the individual samples of each milking, usually within twenty-four hours.

CHARACTER OF RATIONS AND ROUTINE OF THE EXPERIMENTS

The roughage part of the ration during Series I and II consisted of corn silage and alfalfa hay, fed in proportions and amounts intended to meet the maintenance requirements of the cows exactly. In Series III and IV part of the silage and hay was replaced by dried beet pulp which was soaked for ten to twelve hours before feeding. The use of roughage in definite proportions to supply maintenance needs made it possible to feed the concentrates quite closely in proportion to milk production.

Refused feeds were weighed back and the amounts recorded. In a few cases in which cows went off feed, it was necessary to supply temporarily feeds other than the experimental ration, but such changes were also recorded. During Series I, the

experimental rations were interchanged gradually, while in the other series the changes in rations were made abruptly.

In making calculations of the amounts of digestible nutrients in feeds, the average analyses as given by Henry and Morrison (6) were used. The requirements for maintenance and milk production were estimated from an average of values given in the Morrison feeding standard (6).

Series I

Cows 668 and 671 were Holstein-Guernsey crossbred animals which were fed during 1921–1922 on "high oil" and "low oil" rations for four-week periods (period III, five weeks) each period being preceded by a one-week transition period in which the rations were gradually interchanged.

The "low oil" concentrate mixture for periods I and II consisted of 100 pounds of wheat bran and 200 pounds dried beet pulp, while for periods III and IV the proportions of the ingredients were reversed. The "high oil" mixture during periods I and II was composed of high oil corn, grown in plant breeding experiments, and containing about 9.9 per cent ether extract and sunflower seed in equal parts by weight. In periods III and IV, the mixture was made up of 200 pounds of high oil corn, 200 pounds sunflower seed and 300 pounds soybean seed. The feeds were mixed and then ground.

Series II

The check concentrate mixture used in Series II, which was carried out during July and August, 1923, consisted of 200 pounds ground corn, 200 pounds ground oats, 200 pounds wheat bran and 100 pounds linseed oil meal. The "high oil" mixture for this series consisted of 250 pounds "high oil" corn, 100 pounds sunflower seed and 150 pounds soybeans. The mixture was ground. Silage made from "high oil" corn containing about 2 per cent ether extract, was substituted for normal silage during the high oil periods. The animals employed were grade Holstein cows.

Series III

In both Series I and Series II, the concentrates comprising the low oil and high oil mixtures came from different plant sources. It was planned that in Series III and IV, which were carried out during 1924 and 1925, respectively, that the concentrate mixtures being compared should be made up of the same kind of feeds and in as nearly the same proportions as possible, with the exception that the high oil mixtures should contain feeds consisting of ground whole seeds, whereas the low oil mixtures should contain feeds resulting from the extraction of the oil from the same kinds of seeds. In this way soybeans were compared with soybean oil meal, peanuts with peanut oil meal, and flaxseed with linseed oil meal. The concentrate mixtures were so planned for Series III that the percentage of digestible protein remained constant throughout the series, regardless of the content of total digestible nutrients and digestible fat. The mixtures were fed at levels intended to meet the estimated requirements as closely as possible. This was accomplished with a considerable measure of success.

The roughage supplied during Series III was fed at a level intended to meet the maintenance requirements and consisted of 20 pounds of corn silage and 8 pounds alfalfa hay daily per 1000 pounds live weight.

The animals used in Series III and IV were purebred dairy cows, with the exception of cows 6 and 25, which were grades. Several of the cows were employed in both series of experiments.

Precautions were taken in Series III and IV of these experiments to insure a level of protein and total digestible nutrient intake in accord with the animals needs, and to avoid so far as possible, changes in the character of the nutrients supplied. In many previous experiments of this nature, one factor, which may have an important bearing upon the interpretation of the causes of observed changes in the composition of milk, was not controlled. This factor was the energy content of the rations. An oil or feed high in oil was usually added to a basal ration, thus making the high oil ration much higher in energy value

than the basal ration used as a check. It is believed that unless experiments can be carried out in which a comparison is made of rations having iso-dynamic values, and fed at levels which exactly meet the needs of the animals, it cannot be definitely proved that differences in the oil content of rations cause differences in the composition of milk produced. In discussing the specific effects of feeds, Armsby (4) points out that:

> The effects of such feeds as palmnut meal, cocoa meal and cottonseed meal, for example, are reported by different experimenters as favorable, unfavorable or indifferent.
>
> Defective planning of experiments is doubtless responsible for much of this confusion. In many instances the experimenters have simply added the feed to be tested to a light basal ration. . . . . Others, while substituting one feeding stuff for another, have failed to show that the total amount of digestible matter supplied was unchanged. In some extensive investigations, for instance, oil meals and similar feeds have been interchanged in amounts supplying equal quantities of protein without regard to the other ingredients. Under such conditions concordant results could not be expected and one can but agree with Lemmermann and Linkh that the evidence is inconclusive. . . . .

In Series III and IV, therefore, a special effort was made to balance the rations and to adjust frequently the amounts of concentrates fed so that the requirements would be met closely.

Series IV

The plan followed in Series IV was somewhat similar to that of Series III. Each experiment consisted of five periods, each 7 days in length. During periods I, III and V the oil meal concentrate mixture was fed, while during period II, a concentrate mixture in which the ground whole seeds replaced the oil meal was fed.

It was planned to make the oil content of the oil meal rations and whole seeds rations differ as much as possible while still balancing each mixture to meet the requirements of milk production exactly. This difference was naturally greater when seeds rich in oil, such as peanuts, were fed in comparison with

peanut oil meal, and less in the experiments in which seeds only moderately rich in oil, such as soybeans, were fed. During period IV an amount of oil equivalent to the difference in digestible fat content of the oil meal rations fed in periods I and III, and that of the seeds ration fed in period II, was combined with the oil meal concentrate mixture. In making the combination, the starch was omitted or greatly reduced, and in some cases the quantity of beet pulp was reduced also, in order to keep the energy value of the ration as measured by its content of total digestible nutrients at the same level as during the three previous periods. In making the calculations it was assumed that the oil added was entirely digestible.

DISCUSSION OF RESULTS

Series I

The high oil rations seemed to exert very little, if any, effect upon milk yield (table 1). The amounts of total digestible nturients consumed were practically the same during the high oil and low oil periods (figs. 2 and 3).

The percentage butterfat content of the milk was apparently increased somewhat at the beginning of the high oil feeding, but as the periods advanced, the percentage declined. The average test of the milk for the combined yields of the high oil periods was no higher than for the low oil periods (table 1). The procedure followed in determining the butterfat content of the milk (7-day composites) was evidently not sufficiently detailed for following marked changes in the test, if there were such.

Series II

Samples of milk for analysis were taken beginning at the milking following the one at which the change in feed was made.

The percentage of fat and fat yield for the group in period II, during which the high oil ration was fed, were about 8 per cent greater than the averages of periods I and III, during which the herd ration was fed (table 2). The content of total solids showed

the same trend as the fat content, though the increase was not so marked. The percentage of fat in period IV, another high oil period, was also slightly higher than in the periods preceding and following it. Period IV, however, was 18 days in length, and the effect of the high oil feeding upon the test is better seen by examining the productions during different portions of the period, as is done in table 3. Butterfat was determined in the milk of each milking during the first two days of the period and

TABLE 1

Summary of production of cows, Series I

PERIOD*	cow 668				cow 671			
	Ration	Milk	Fat	Per cent fat	Ration	Milk	Fat	Per cent fat
		pounds	*pounds*			*pounds*	*pounds*	
Preliminary....	Herd	631.6	32.3	5.1	Herd	814.7	34.0	4.2
I.....	High oil	743.7	34.3	4.6	Low oil	801.4	35.9	4.5
II.....	Low oil	690.8	31.0	4.5	High oil	838.8	32.2	3.8
III.....	High oil..	663.5	31.2	4.7	Low oil	796.5	31.2	3.9
IV.....	Low oil	578.9	24.7	4.3	High oil	750.5	28.5	3.8
Subsequent....	Herd	672.9	30.3	4.5	Herd	865.5	31.2	3.6

	MILK	FAT	PER CENT FAT
	pounds	*pounds*	
Total production of both cows during high oil periods.....	2,996.5	126.2	4.21
Total production of both cows during low oil periods.....	2,867.6	122.8	4.28

* Periods were 4 weeks in length, except period II, which was 5 weeks. For comparison, period II has been corrected to a basis of 4 weeks.

in composite samples representing the other portions of the period.

The average test for the first two days of period IV was nearly 16 per cent higher than the average for the whole period, and 18 per cent higher than the three periods of herd ration feeding. The next three days' test showed a sharp drop. The high oil feeds seemed to have lost their effect upon the test by the close of the fifth day.

TABLE 2

Summary of production of cows, Series II, 1923

PERIOD	RATION	COW	TOTAL SOLIDS		MILK	FAT	TEST
			pounds	per cent	pounds	pounds	
I (10 days)	Herd	571	39.19	12.29	318.9	11.07	3.47
		574	56.43	12.07	467.5	15.95	3.41
		575	41.61	11.55	360.0	11.19	3.11
		576	43.42	11.40	380.6	11.35	2.98
Total			180.65		1,527.0	49.56	
Average per day			18.07	11.83	152.7	4.96	3.25
II (3 days)	High oil	571	11.25	12.18	92.4	3.23	3.50
		574	16.18	12.16	133.1	4.86	3.65
		575	11.81	11.89	99.3	3.39	3.41
		576	12.13	11.65	104.1	3.45	3.32
Total			51.37		428.9	14.93	
Average per day			17.12	11.98	143.0	4.98	3.48
III (12 days)	Herd	571	43.55	11.95	364.5	11.81	3.24
		574	61.23	11.89	515.0	17.22	3.34
		575	44.25	11.43	387.1	11.97	3.09
		576	47.41	11.45	413.9	12.53	3.03
Total			196.44		1,680.5	53.53	
Average per day			16.37	11.69	140.1	4.46	3.19
IV (18 days)	High oil	571	62.03	12.36	501.7	17.50	3.49
		574	72.73	11.91	610.6	21.12	3.46
		575	61.70	11.72	526.3	17.06	3.24
		576	70.06	11.65	601.0	17.93	2.98
Total			266.52		2,239.6	73.61	
Average per day			14.81	11.90	124.4	4.09	3.29
V. (21 days)*	Herd	571			551.7	18.12	3.29
		574			722.4	24.28	3.36
		575			627.3	19.44	3.10
		576			658.7	20.40	3.10
Total					2,560.1	82.24	
Average per day					121.9	3.92	3.21

* Percentage of fat determined by Babcock method during period V.

TABLE 3

Detailed production of Period IV, Series II, 1923

COW	MILK	SOLIDS		FAT	TEST
First 2 days					
	pounds	*pounds*	*per cent*	*pounds*	
571	59.0	7.26	12.30	2.100	3.56
574	57.9	7.73	13.35	2.876	4.97
575	60.2	7.02	11.66	2.066	3.43
576	68.4	8.08	11.81	2.3505	3.37
Total	245.5	30.09		9.347	
Daily average	122.8	15.05	12.26	4.674	3.81
Days 3, 4, 5					
571	86.1	10.87	12.62	3.168	3.68
574	91.6	10.91	11.91	3.207	3.50
575	86.7	10.19	11.75	2.905	3.35
576	102.6	11.86	11.56	3.016	2.94
Total	367.0	43.83		12.296	
Daily average	122.3	14.61	11.94	4.099	3.35
Days 6, 7, 8					
571	88.1	10.73	12.18	2.969	3.37
574	107.0	12.58	11.76	3.306	3.09
575	88.1	10.43	11.84	2.908	3.30
576	102.4	11.81	11.53	3.021	2.95
Total	385.6	45.55		12.204	
Daily average	128.5	15.18	11.81	4.068	3.17
Days 9, 10, 11					
571	86.7	10.72	12.36	3.009	3.47
574	109.9	12.84	11.68	3.440	3.13
575	88.0	10.40	11.82	2.834	3.22
576	98.4	11.65	11.84	2.943	2.99
Total	383.0	45.61		12.226	
Daily average	127.7	15.20	11.91	4.075	3.19
Days 12 to 18					
571	181.8	22.45	12.35	6.253	3.44
574	244.2	28.67	11.74	8.293	3.40
575	203.3	23.66	11.14	6.342	3.12
576	229.2	26.66	11.63	6.646	2.90
Total	858.5	101.44		27.534	
Daily average	122.6	14.49	11.82	3.933	3.21

The differences in oil content of the rations being compared were relatively small. The calculated daily amounts of digestible fat consumed by the four cows in the first four periods were 3.8, 6.1, 3.7 and 5.7 pounds, respectively. In spite of the small differences, there was a distinct effect of the rations upon the test of the milk. The amounts of digestible protein and total digestible nutrients consumed met the estimated requirements of these substances closely (fig. 4).

A comparison of the Babcock and Roese-Gottlieb methods of determining the percentage butterfat content of milk was made during Series II. It was found that the Babcock method gave results slightly higher (0.04 to 0.13 per cent fat for the different cows) than the Roese-Gottlieb method (table 4).

Series III

This series comprised three feeding trials of three weeks each, covering a total time of about ten weeks. Period I, during which the soybean oil meal ration was fed, was extended to 13 days in order that the cows might become better accustomed to the feed, and also on account of the recent freshening of some of them. The other periods were seven days in length and the records of milk production coincide with the feeding periods.

The number of days the cows had been in milk at the beginning of Series III was as follows: Cow 262, 2 days; cow 270, 49 days; cow 300, 7 days; cow 303, 5 days; cow 316, 309 days; cow 323, 13 days. Cows 6 and 25 had been in milk 87 days and 102 days, respectively, at the beginning of the feeding trials reported for them under experiment B.

The increases in test obtained by replacing soybean oil meal in the concentrate mixture with soybeans were not large, and in one case, that of cow 262, the results were negative (table 5). The results obtained with cow 262 are not conclusive, however, because she continued to increase in milk production throughout both experiments A and B and failed to consume enough nutrients to meet her requirements. The recent freshening of several of the cows seemed to be a factor of minor importance in determining the extent to which the ration affected the test.

TABLE 4

Comparison of Babcock and Roese-Gottlieb methods for determination of butterfat in milk

SAMPLE NUMBER	RATION	COW 571		COW 574		COW 575		COW 576	
		B‡	R§	B‡	R§	B‡	R§	B‡	R§
1*	Herd	3.7	3.69	3.6	3.58	3.1	3.09	3.0	2.98
2*	Herd	3.5	3.32	3.4	3.29	3.25	3.11	3.0	2.96
3*	Herd	3.5	3.38	3.6	3.45	3.2	3.06	3.2	3.03
4†	Herd	3.8	3.67	3.15	3.00	3.4	3.27	3.1	2.99
5†	Herd	3.6	3.47	3.45	3.31	3.5	3.34	2.9	2.89
6†	High oil	3.95	3.85	4.05	4.02	3.95	3.84	3.6	3.52
7†	High oil	3.4	3.19	3.9	3.84	3.2	2.98	3.0	2.84
8†	High oil	3.7	3.56	3.9	3.74	3.9	3.77	2.95	3.09
9†	High oil	3.9	3.66	3.5	3.24	3.35	3.16	3.2	3.15
10†	High oil	4.1	4.11	3.7	3.60	3.8	3.62	3.6	3.52
11†	High oil	3.05	2.79	3.7	3.54	3.5	3.28	3.0	3.83
12†	Herd	4.1	4.10	2.9	2.82	2.8	2.75	2.6	2.56
13†	Herd	3.1	3.01	3.2	3.10	3.1	3.01	2.8	2.55
14†	Herd	3.3	3.58	2.9	2.88	3.0	3.08	3.3	3.34
15†	Herd	3.2	3.17	3.3	3.30	3.05	3.08	2.5	2.39
16*	Herd	3.4	3.19	3.5	3.30	3.3	3.06	3.3	3.13
17*	Herd	3.3	3.19	3.5	3.28	3.4	3.21	3.1	3.03
18*	Herd	3.4	3.18	3.6	3.50	3.1	3.04	3.2	3.21
19†	Herd	3.6	3.58	3.5	3.46	3.2	3.09	3.0	2.98
20†	Herd	3.2	3.08	4.4	4.38	3.35	3.34	2.9	2.78
21†	High oil	3.1	3.09	4.3	4.25	3.4	3.22	3.8	3.76
22†	High oil	3.7	3.64	5.1	5.15	3.6	3.50	3.6	3.53
23†	High oil	4.2	4.11	5.0	4.92	3.6	3.28	3.1	3.07
24†	High oil	3.5	3.39	5.7	5.76	3.7	3.65	3.2	3.13
25*	High oil	3.85	3.68	3.6	3.50	3.5	3.35	3.1	2.94
26*	High oil	3.5	3.37	3.2	3.09	3.5	3.30	3.1	2.95
27*	High oil	3.5	3.47	3.2	3.13	3.25	3.22	3.05	2.99
28*	High oil	3.5	3.44	3.3	3.40	3.3	3.12	2.9	2.90
Average.....		3.55	3.46	3.72	3.61	3.37	3.24	3.11	3.07

* Composite sample preserved by formalin.

† Sample of single milking—no preservative.

‡ Babcock method, average of 2 determinations.

§ Roese-Gottlieb method, average of 2 determinations.

however, for cows 270 and 316, which had been in milk for a longer time, showed increases in test only slightly greater than cows 300 and 303.

It is believed that the failure of the soybean ration to cause a

TABLE 5

Summary of results

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT YIELD
Series III, experiment A. Comparison of effect of soybean oil meal and soybeans on the percentage of fat in milk					
Cow 262 (Guernsey)					
	pounds	*pounds*		*per cent*	*per cent*
Soybean oil meal*	257.0	12.950	5.18		
Soybeans	288.5	14.774	5.12	−2.1	−1.7
Soybean oil meal	324.0	17.111	5.28		
Cow 270 (Guernsey)					
Soybean oil meal*	167.2	9.671	5.87		
Soybeans	165.4	10.155	6.14	6.1	5.2
Soybean oil meal	168.8	9.637	5.70		
Cow 31¢ (Holstein)					
Soybean oil meal*	276.5	9.880	3.57		
Soybeans	266.2	9.643	3.64	4.6	−1.5
Soybean oil meal	286.1	9.703	3.39		
Cow 300 (Guernsey)					
Soybean oil meal*	250.0	11.416	4.57		
Soybeans	240.8	11.657	4.80	7.3	5.2
Soybean oil meal	245.6	10.752	4.37		
Cow 303 (Guernsey)					
Soybean oil meal*	122.7	6.579	5.36		
Soybeans	135.0	7.099	5.26	3.2	2.3
Soybean oil meal	150.2	7.299	4.83		
Cow 323 (Ayrshire)					
Soybean oil meal*	173.1	8.844	5.11		
Soybeans	176.3	8.699	4.93	3.0	9.3
Soybean oil meal	158.8	7.078	4.46		
Average periods 1 and 3:					
Soybean oil meal, 6 cows	1,290.5	60.460	4.69		
Soybeans, 6 cows	1,271.2	62.027	4.88	4.1	2.6

* Period I was 13 days in length, but for comparative purposes has been calculated to a basis of 7 days.

TABLE 3—*Continued*

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT YIELD
Series III, experiment B. Comparison of effect of linseed oil meal with flaxseed meal on the percentage of fat in milk					
Cow 262 (Guernsey)					
	pounds	*pounds*		*per cent*	*per cent*
Linseed oil meal	331.9	15.782	4.76		
Flaxseed meal	333.9	16.146	4.84	1.2	1.0
Linseed oil meal	336.8	16.193	4.81		
Cow 270 (Guernsey)					
Linseed oil meal	163.5	9.281	5.68		
Flaxseed meal	172.2	10.924	6.34	12.0	15.4
Linseed oil meal	171.0	9.650	5.64		
Cow 270 (Guernsey)					
Linseed oil meal	141.3	8.126	5.75		
Flaxseed meal	151.3	9.283	6.13	11.4	10.9
Linseed oil meal	163.6	8.614	5.26		
Cow 316 (Holstein)					
Linseed oil meal	297.2	10.358	3.49		
Flaxseed meal	293.8	11.142	3.80	7.6	11.2
Linseed oil meal	270.4	9.659	3.57		
Cow 300 (Guernsey)					
Linseed oil meal	232.7	10.159	4.37		
Flaxseed meal	227.5	11.343	4.98	11.7	13.2
Linseed oil meal	217.4	9.883	4.55		
Cow 303 (Guernsey)					
Linseed oil meal	145.0	6.778	4.67		
Flaxseed meal	146.6	7.341	5.01	9.2	6.4
Linseed oil meal	155.7	7.018	4.51		
Cow 323 (Ayrshire)					
Linseed oil meal	153.8	6.834	4.44		
Flaxseed meal	137.0	7.319	5.34	21.9	14.1
Linseed oil meal	138.6	5.991	4.32		

TABLE 5—*Concluded*

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT YIELD
	pounds	*pounds*		*per cent*	*per cent*
Series III experiment B. Comparison of effect of linseed oil meal with flaxseed meal on the percentage of fat in milk—Continued					
Cow 6 (Grade Holstein)					
Flaxseed meal†	219.9	8.115	3.69		
Linseed oil meal	217.7	7.031	3.23		
Flaxseed meal	192.0	7.332	3.82	14.2	0.7
Linseed oil meal	217.4	7.529	3.46		
Cow 25 (Grade Holstein)					
Flaxseed meal†	263.5	8.198	3.11		
Linseed oil meal	252.4	7.030	2.78		
Flaxseed meal	236.6	7.648	3.23	11.0	5.0
Linseed oil meal	247.5	7.544	3.04		
Average periods 1 and 3:					
Linseed oil meal, 9 cows	1,927.0	81,730	4.24		
Flaxseed meal, 9 cows	1,890.9	88.478	4.68	10.4	8.3
Series III, experiment C. Comparison of effect of peanut oil meal and peanuts on the percentage of fat in milk					
Cow 316 (Holstein)					
Peanut oil meal	262.1	9.573	3.65		
Peanuts	276.9	9.777	3.53	3.1	1.4
Peanut oil meal	297.3	9.719	3.20		
Cow 300 (Guernsey)					
Peanut oil meal	195.9	8.596	4.39		
Peanuts	194.0	9.456	4.87	5.6	12.4
Peanut oil meal	170.2	8.224	4.83		
Cow 303 (Guernsey)					
Peanut oil meal	155.9	6.891	4.42		
Peanuts	148.6	7.233	4.87	14.1	6.1
Peanut oil meal	163.9	6.745	4.12		
Cow 323 (Ayrshire)					
Peanut oil meal	139.1	6.106	4.39		
Peanuts	135.3	6.888	5.09	18.6	15.7
Peanut oil meal	138.3	5.802	4.19		
Average periods 1 and 3:					
Peanut oil meal, 4 cows	761.4	30.828	4.05		
Peanuts, 4 cows	754.8	33.354	4.42	9.1	8.2

† Preliminary period.

greater effect upon the test was due to the comparatively small difference in the oil content of the soybean oil meal mixture and that containing the ground soybeans.

When flaxseed meal was substituted for linseed oil meal in amounts such that the concentrate mixture was as closely balanced to meet production requirements as before, greater effects upon the test were secured than with any other like substitution. The increases were as great as 21.9 per cent with an average for the nine records of eight cows of 11 per cent (table 5, experiment B). If cow 262 be omitted, the average would be about 12 per cent. The increase in the test was responsible for an increased fat yield for the week about 8 per cent greater than the average for the preceding and following weeks, although some of the increased fat yields were as much as 14 per cent. The milk yield was somewhat lower during the flaxseed meal period, the average for the group being 4.4 pounds per cow for the week, or about 0.6 pound per day.

The peanut oil meal and peanut mixtures were not consumed readily by some cows, and were refused entirely by others. Cow 270 was, therefore, again fed the rations used in experiment B, and her records included under that experiment. The substitution of peanuts for peanut oil meal gave increases in test of as much as 18 per cent, with an average of 9 per cent for the four cows (table 5, experiment C). Fat yields were also increased about 8 per cent.

Neither the peanut oil meal nor peanut mixtures were readily consumed, which is believed to be responsible for decreased milk production in several cases and failure to secure a greater effect upon the test.

The amounts of digestible nutrients consumed compared with the estimated requirements are shown in figures 5 and 10.

One of the interesting observations made during this series was that the test is not affected at once when the ration is changed from low oil to high oil, but that there is a latent period which seems to vary in length from 12 to 36 hours. Part of the records secured in Experiment B have been calculated in different ways in order to illustrate this point (table 6). It was

TABLE 6

Effect on production and test of changing from linseed oil meal ration to flaxseed ration

Records calculated in different ways*

COW NUMBER	CHECK PERIOD (4 DAYS PRECEDING FEED CHANGE)			RECORDS FOR ONE DAY BEGINNING WITH FEED CHANGE			RECORDS BEGINNING ONE MILKING AFTER FEED CHANGE						RECORDS FOR 2 DAYS BEGINNING 2 MILKINGS AFTER FEED CHANGE				
							First day			First and second days							
	Average daily milk	Average daily fat	Fat	Milk	Fat		Milk	Fat		Average daily milk	Average daily fat	Fat	Average daily milk	Average daily fat	Fat	Increase in per cent fat	Increase in test per cent
	pounds	*pounds*	*per cent*	*pounds*	*pounds*	*per cent*	*pounds*	*pounds*	*per cent*	*pounds*	*pounds*	*per cent*	*pounds*	*pounds*	*per cent*		
300	33.3	1.468	4.41	31.9	1.356	4.25	33.3	1.419	4.26	33.1	1.522	4.57	31.8	1.546	4.86	+0.45	10.2
262	47.2	2.145	4.55	44.7	2.034	4.55	48.3	2.144	4.44	47.3	2.275	4.81	47.5	2.294	4.84	+0.29	6.4
270	23.5	1.395	5.94	22.9	1.365	5.95	24.5	1.541	6.29	24.4	1.609	6.59	24.5	1.643	6.70	+0.76	12.8
270	20.6	1.176	5.71	20.6	1.175	5.70	21.0	1.333	6.35	21.5	1.395	6.50	21.8	1.462	6.70	+0.99	17.3
303	19.7	0.938	4.75	21.1	0.982	4.65	20.1	0.993	4.94	19.4	0.978	5.04	19.9	0.996	5.00	+0.25	5.3
316	42.2	1.465	3.47	43.4	1.504	3.46	42.5	1.528	3.59	42.5	1.638	3.86	42.7	1.685	3.95	+0.48	13.8
323	21.6	0.954	4.42	22.4	0.969	4.33	22.6	0.968	4.28	22.5	1.044	4.64	22.9	1.123	4.89	+0.47	10.7
Average...	29.7	1.363	4.59	29.6	1.341	4.53	30.3	1.418	4.68	30.1	1.494	4.98	30.2	1.536	5.09	+0.50	10.9
Average gain or loss.....				−0.1	−0.022		+0.6	+0.055		+0.4	+0.131	+0.39	+0.5	0.173	+0.50	+0.50	
Increase in test, per cent...						−1.3			2.0			8.5					10.9

* Cows milked twice daily, except cow 316. Records for cow 316 calculated for same periods as other cows.

found that when the milk yields and fat yields were computed for one day beginning with the milking coinciding with the change in feed, that the test was no higher than in the four day check period preceding the change. Records for one day and for two days beginning one milking after the feed change were tabulated. The first days' test showed an increase of 2 per cent above that of the check period, while the increase in test for the two days was 8.5 per cent, thus indicating that the change during the second day was greater than during the first. When two milkings after the feed change were omitted, the records for the two following days showed an increase in test of nearly 11 per cent.

Another point brought out in these studies was that the high oil ration has a residual effect lasting from 12 to 24 hours or more (table 7). In the latter table, the records are given by periods beginning one day after the changes in rations were made. Further studies of the latent effects of feed are in progress.

In view of the increases in test obtained in these experiments in which concentrate mixtures containing about 11.5 per cent digestible protein were fed, the question was raised as to whether similar increases could be obtained with rations higher in protein. Accordingly, cow 270 was fed for three additional periods of seven days each. During the first and third periods, a "test cow mixture," consisting of corn, oats, wheat bran, linseed oil meal, cottonseed meal, soybean oil meal and gluten meal, and containing about 17 per cent digestible protein, was fed. During the second period, flaxseed meal was substituted for a part of the test mixture. The detailed production records are shown in table 7.

The data show that the daily yield of fat and the test were approximately 16 per cent greater during the flaxseed period than during the test ration periods.

Series IV

The procedure during Series IV was similar to that of the preceding series, except that each experiment was extended to include two more weeks, during one of which oil plus oil meal

TABLE 7

The effect of feeding flaxseed when the test ration is used as the basic ration. Flaxseed substituted for part of test ration during flaxseed period

Guernsey cow no. 270

	MILK YIELD		FAT	FAT YIELD	
	Per milking	Daily		Per milking	Daily
Test ration					
	pounds	*pounds*	*per cent*	*pounds*	*pounds*
May 10—A	11.0		5.3	0.583	
P	10.5	21.5	5.4	0.567	1.150
May 11—A	11.3		5.6	0.633	
P	10.2	21.5	5.4	0.551	1.184
May 12—A	11.6		5.3	0.615	
P	10.2	21.8	5.6	0.571	1.186
May 13—A	12.5		5.4	0.675	
P	12.7	25.2	5.3	0.673	1.348
May 14—A	13.4		5.6	0.750	
P	9.6	23.0	5.8	0.557	1.307
May 15—A	11.9		5.8	0.690	
P	10.0	21.9	6.0	0.600	1.290
May 16—A	11.0		4.7	0.517	
P	10.0	21.0	6.5	0.650	1.167
Total		155.9			8.632
Average		22.3	5.56		1.277
Flaxseed ration					
	pounds	*pounds*	*per cent*	*pounds*	*pounds*
May 17—A	12.1		5.7	0.690	
P	9.9	22.0	6.2	0.614	1.304
May 18—A	11.2		6.0	0.672	
P	9.8	21.0	6.3	0.617	1.289
May 19—A	11.5		6.3	0.725	
P	9.6	21.1	6.8	0.653	1.378
May 20—A	12.6		6.4	0.806	
P	10.3	22.9	7.4	0.762	1.568
May 21—A	11.8		6.9	0.814	
P	10.5	22.3	7.4	0.777	1.591
May 22—A	11.6		5.9	0.684	
P	9.9	21.5	7.2	0.713	1.397
May 23—A	12.2		6.7	0.817	
P	10.0	22.2	7.3	0.730	1.547
Total		153.0			10.074
Average		21.9	6.58		1.439

TABLE 7—*Continued*

	MILK YIELD		FAT	FAT YIELD	
	Per milking	Daily		Per milking	Daily
Test ration					
May 24—A	10.4		6.5	0.676	
P	9.6	20.0	6.9	0.661	1.337
May 25—A	12.5		6.5	0.813	
P	10.7	23.2	6.0	0.642	1.455
May 26—A	10.8		5.6	0.605	
P	9.8	20.6	5.3	0.519	1.124
May 27—A	12.5		5.4	0.675	
P	9.9	22.4	5.4	0.535	1.210
May 28—A	11.8		5.2	0.614	
P	10.1	21.9	5.8	0.586	1.200
May 29—A	12.0		5.6	0.672	
P	9.2	21.2	5.5	0.506	1.178
May 30—A	12.3		5.7	0.701	
P	9.9	22.2	5.6*	0.554	1.256
Total		151.5			8.760
Average		21.6	5.78		1.251

	MILK	FAT	
	pounds	*pounds*	*per cent*
Average daily production on test ration	22.0	1.242	5.66
Average daily production on flaxseed ration	21.9	1.439	6.58

Increase in yield of fat, per cent	15.9
Increase in test, per cent	16.3

* Sample lost. Average test of three previous p.m. milkings used.

characterized the ration. The rations differed from those of Series III in being less bulky and thus more readily consumed. Corn, oats and corn starch were used instead of the large quantities of beet pulp, these feeds being used to provide energy and to keep at a low level the protein and oil content of that part of the concentrate mixture other than the feeds being compared.

On account of the differences in the requirements for the production of milk containing different percentages of fat, two separate concentrate mixtures were formulated for each period, one being calculated for Holsteins and Ayrshires, and the other

TABLE 8

Summary of results

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT
Series IV, experiment A. Comparison of effect of linseed oil meal, flaxseed meal and linseed oil meal plus linseed oil on the percentage of fat in milk					
Cow 299 (Jersey)					
	pounds	*pounds*		*per cent*	*per cent*
Linseed oil meal	200.7	9.988	4.98		
Flaxseed meal	204.0	10.475	5.13	8.7	6.9
Linseed oil meal	215.9	9.606	4.45		
Linseed oil meal + linseed oil	215.0	10.185	4.74	9.5	10.9
Linseed oil meal	208.3	8.768	4.21		
Cow 340 (Holstein)					
Linseed oil meal	245.7	7.485	3.05		
Flaxseed meal	233.5	6.988	2.99	0.7	−0.01
Linseed oil meal	224.3	6.493	2.89		
Linseed oil meal + linseed oil	221.1	7.022	3.18	15.6	13.3
Linseed oil meal	226.4	5.905	2.61		
Cow 288 (Holstein)					
Linseed oil meal	352.0	11.415	3.24		
Flaxseed meal	341.7	10.367	3.03	1.2	−0.5
Linseed oil meal	342.0	9.412	2.75		
Linseed oil meal + linseed oil	342.3	10.527	3.08	13.0	15.5
Linseed oil meal	327.0	8.819	2.70		
Cow 288					
Linseed oil meal	314.9	10.043	3.189		
Linseed oil meal	312.3	9.619	3.080		
Flaxseed meal	306.5	10.400	3.39	10.1	9.7
Linseed oil meal	300.4	9.077	3.02		
Linseed oil meal	303.8	9.181	3.02		
Average periods 1 and 3:					
Linseed oil meal, 3 cows	790.3	27.200	3.44		
Flaxseed meal, 3 cows	779.2	27.830	3.57	3.7	2.3
Average periods 3 and 5:					
Linseed oil meal, 3 cows	772.0	24.502	3.17		
Linseed oil meal + linseed oil, 3 cows	778.4	27.734	3.56	12.3	13.2

TABLE 8—*Continued*

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT
Series IV, experiment B. Comparison of effect of peanut oil meal, peanuts, and peanut oil meal plus peanut oil on the percentage of fat in milk					
Cow 270 (Guernsey)					
	pounds	*pounds*		*per cent*	*per cent*
Peanut oil meal	204.9	10.424	5.087		
Peanuts	201.4	11.415	5.668	13.6	8.6
Peanut oil meal	216.4	10.590	4.89		
Peanut oil meal + peanut oil	193.0	10.978	5.69	16.0	7.8
Peanut oil meal	198.5	9.773	4.92		
Cow 340 (Holstein)					
Peanut oil meal	233.5	6.551	2.805		
Peanuts	214.8	7.067	3.290	14.9	8.1
Peanut oil meal	223.2	6.518	2.92		
Peanut oil meal + peanut oil	214.7	6.772	3.15	12.3	6.4
Peanut oil meal	232.4	6.266	2.69		
Cow 299 (Jersey)					
Peanut oil meal	221.6	10.167	4.588		
Peanuts	206.8	10.051	4.860	9.7	3.0
Peanut oil meal	219.2	9.357	4.27		
Peanut oil meal + peanut oil	213.5	10.408	4.87	16.7	14.5
Peanut oil meal	216.4	8.819	4.07		
Cow 334 (Jersey)					
Peanut oil meal	232.0	11.377	4.904		
Peanuts	234.9	11.481	4.888	7.0	1.2
Peanut oil meal	264.7	11.312	4.27		
Peanut oil meal + peanut oil	238.2	11.283	4.73	5.3	−1.8
Peanut oil meal	246.3	11.657	4.73		
Cow 323 (Ayrshire)					
Peanut oil meal	226.6	10.532	4.648		
Peanuts	224.7	9.690	4.312	−0.9	−0.4
Peanut oil meal	222.5	9.003	4.07		
Peanut oil meal + peanut oil	219.3	9.248	4.26	14.8	7.2
Peanut oil meal	224.8	8.244	3.66		
Average periods 1 and 3:					
Peanut oil meal, 5 cows	1,132.3	47.916	4.23		
Peanuts, 5 cows	1,082.6	49.704	4.59	8.5	3.7
Average periods 3 and 5:					
Peanut oil meal, 5 cows	1,132.2	45.770	4.04		
Peanut oil meal + peanut oil	1,078.7	48.689	4.51	11.64	6.4

TABLE 8—*Continued*

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT
Series IV, experiment C. Comparison of effect of soybean oil meal, soybeans, and soybean oil meal plus soybean oil on the percentage of fat in milk					
Cow 270 (Guernsey)					
	pounds	*pounds*		*per cent*	*per cent*
Soybean oil meal	193.5	9.637	4.980		
Soybeans	178.7	9.652	5.401	4.4	0.1
Soybean oil meal	179.6	9.656	5.376		
Soybean oil meal + soybean oil	167.0	9.175	5.494	1.5	−5.2
Soybean oil meal	167.3	9.699	5.797		
Cow 340 (Holstein)					
Soybean oil meal	213.8	6.123	2.864		
Soybeans	209.9	6.249	2.977	1.1	−5.1
Soybean oil meal	233.7	7.055	3.019		
Soybean oil meal + soybean oil	233.1	6.649	2.852	−5.7	−4.7
Soybean oil meal	227.6	6.899	3.031		
Cow 288 (Holstein)					
Soybean oil meal	292.4	8.994	3.076		
Soybeans	295.9	9.541	3.224	−3.1	6.1
Soybean oil meal	283.3	8.995	3.175		
Soybean oil meal + soybean oil	300.7	9.188	3.056	−3.0	1.1
Soybean oil meal	290.5	9.174	3.158		
Cow 299 (Jersey)					
Soybean oil meal	212.5	9.519	4.480		
Soybeans	195.4	8.740	4.473	−4.7	−7.7
Soybean oil meal	191.4	9.422	4.923		
Soybean oil meal + soybean oil	197.9	9.485	4.793	−0.8	1.1
Soybean oil meal	196.8	9.341	4.746		
Cow 323 (Ayrshire)					
	pounds	*pounds*		*per cent*	*per cent*
Soybean oil meal	203.3	7.722	3.798		
Soybeans	195.7	8.012	4.094	5.7	4.3
Soybean oil meal	193.5	7.643	3.950		
Soybean oil meal + soybean oil	175.3	6.964	3.973	−8.0	−11.1
Soybean oil meal	173.6	8.021	4.620		

TABLE 8—*Concluded*

PERIOD	MILK, 7 DAYS	FAT, 7 DAYS	AVERAGE TEST (PER CENT FAT)	INCREASE IN TEST	INCREASE IN FAT
Series IV, experiment C. Comparison of effect of soybean oil meal, soybeans and soybean oil meal plus soybean oil on the percentage of fat in milk					
Cow 323 (Ayrshire)—*Continued*					
Average periods 1 and 3:				*per cent*	*per cent*
Soybean oil meal, 5 cows	1,098.5	42.383	3.86		
Soybeans, 5 cows	1,075.6	42.194	3.92	1.6	−0.4
Average periods 3 and 5:					
Soybean oil meal, 5 cows	1,068.7	42.953	4.02		
Soybean oil meal + soybean oil, 5 cows	1,074.0	41.461	3.86	−4.0	−3.5

for Jerseys and Guernseys. The experiments began shortly after the cows had calved, the stage of lactation being as follows: Cow 299, 44 days; cow 340, 34 days; cow 288, 37 days; cow 270, 37 days; cow 334, 10 days; cow 323, 21 days.

Since experiments of Series III had shown a latent period and a residual effect of the high oil feeding, the records of milk production during Series IV were calculated for periods beginning one day after the changes in the rations were made.

The comparison of the oil meal feeds with the kind of seeds from which the oil meals were derived gave results similar to those of Series III (table 8). Flaxseed meal, however, did not affect the test to so great an extent as before. During period IV of experiment A, linseed oil was administered to cows 299, 340 and 288, in amounts of 1.5, 1 and 1.7 pounds, respectively. The linseed oil apparently had greater effects upon the test than the feeding of an equal amount of oil in the flaxseed meal rations. The few records obtained, however, can not be taken as indicating the exact extent of the effects upon the test to be expected from changes of this sort in the rations.

The comparison of peanut oil meal and peanuts was more satisfactory than before because the feeds were more palatable. The peanut oil meal was quite readily consumed, but the peanuts, at first fed raw and later roasted, were not eaten so readily as the oil meal. The increases in test due to the peanut feeding (table

8, experiment B) were similar to those obtained in Series III, while the peanut oil gave slightly greater increases. The oil was fed in amounts ranging from 1.6 to 2.6 pounds per head daily.

The comparison of soybean oil meal with soybeans, and the addition of soybean oil to soybean oil meal concentrate mixtures, showed practically no effect of high oil rations upon the test of milk. It is believed that the primary reason for this as suggested above, was the small differences between the low oil and high oil rations. While some of the cows received as much as 2.5 to 3.0 pounds of digestible fat daily during the high oil periods in experiments A and B, the amounts were not over 1.4 pounds daily during the soybean comparisons. The amounts of soybean oil fed ranged from 0.3 to 0.56 pound daily.

A factor which seemed to influence feed consumption and milk yields during experiment C, although it may have had no influence upon the test, was the unusually hot weather for the season of the year (May 2 to June 6, 1925).

Increases in test like those obtained as a result of the flaxseed and peanut feeding would be of much significance if obtained during the taking of an "official test," because the amount of butterfat credited to a cow might be materially increased as a result of the higher fat percentages. Perhaps of even greater importance is the higher percentage of fat which might be obtained during a one-day or two-day "semi-official test," for the average percentage of fat obtained during the one-day or two-day period forms the basis for the calculation of the butterfat yields for an entire month. A cow might thus be credited with a yield of fat greatly in excess of her actual production.

Another point of interest in connection with the making of official tests, is the effect of linseed oil upon the test of the milk. Linseed oil is sometimes administered as a laxative or physic, and might be so used during an official test. It is shown in these experiments that the consumption of linseed oil (given by mouth) increased the test of the milk.

These series of experiments indicate that the composition of milk can be materially influenced by changes in the feed which

increase the amount of digestible fat or oil. The feeding of oils extracted from seeds of different plants had an effect upon the test of the milk similar to that produced by feeding the seeds. It is believed that possible stimulating effects due to changes in protein intake and to the introduction of feeds from different plant sources were absent from the experiments of Series III and IV.

Further, in Series IV, differences in the energy content of the rations, calculated in terms of digestible nutrients, were largely avoided, so that increases in the test of the milk could not be due to a stimulating effect caused by a large surplus of energy above the requirements. It is logical to conclude, therefore, that the factor causing changes in the percentage fat content of the milk was the differences in oil content of the rations compared.

PALATABILITY OF FEEDS

Several of the mixtures fed were not relished, making it necessary to weigh and account for the orts or to change the character of the rations. Production was not so liberal on some rations as it might have been, had the feed been consumed more freely.

Difficulty was encountered in Series III in securing complete consumption of the concentrate mixtures. This was partly due to great bulkiness caused by the large quantities of soaked beet pulp, although the lack of palatability of some of the feeds was also responsible.

The peanut oil meal used in Series III had a sharp, burned odor and taste and was refused by some cows, while in Series IV it was of much better quality. The peanuts fed were shelled No. 2 Virginia peanuts, and were fed both raw and roasted, ground and unground. Some cows refused to eat peanuts, while the ones which did eat them seemed to relish them roasted better than in the raw state. As many as 6.5 pounds of peanuts were consumed per cow daily.

Soybeans did not prove as palatable as the soybean oil meal. About 4 pounds of soybeans daily per cow was the maximum amount fed.

Flaxseed meal was eaten readily by all the cows, and as much

as 45 pounds weekly was fed without apparent disturbance of the body functions.

Peanut oil and soybean oil were consumed completely when mixed with the concentrates, but linseed oil was refused and was given as a drench.

SUMMARY AND CONCLUSIONS

Experiments were carried out to determine the effect of rations high in oil content upon the composition of milk, with particular reference to its percentage fat content (test).

The general plan of procedure followed was to feed rations low in oil content and high in oil content in alternate periods, and to compare the results obtained during a high oil period with the average of the results of the low oil periods immediately preceding and following it.

The feeding of mixtures of sunflower seed, soybean seed and high oil corn did not seem to stimulate milk production and effects upon the test, if any, were not evidenced in weekly composite samples of the milk.

In the second series of experiments, mixtures of sunflower seed, high oil corn and soybean seed caused increases in the test of the milk of 8 to 18 per cent as compared to the tests during periods when the usual herd ration was fed. Slight changes in the percentage of total solids followed in the same direction as the test.

In the third series, all concentrate mixtures contained the same percentage of digestible protein, and the mixtures were fed at a level intended to meet the estimated requirements closely. The mixtures fed during periods I and III of experiments A, B and C contained soybean oil meal, linseed oil meal and peanut oil meal, respectively, while during period II of the same experiments the corresponding oil meals were replaced by soybeans, flaxseed and peanuts. The increases in test secured as a result of the soybean feeding compared to the soybean oil meal periods were small or negative, while flaxseed gave increases of as much as 20 per cent, with an average of over 10 per cenfor nine records, as compared with linseed oil meal. The peat

nuts gave increases of as much as 18 per cent, with an average of 9 per cent for four cows, as compared with peanut oil meal. Fat yields were increased by both flaxseed and peanuts to the extent of about 8 per cent.

In Series IV, the rations were carefully balanced and fed to meet requirements closely. As in the preceding series, a com-

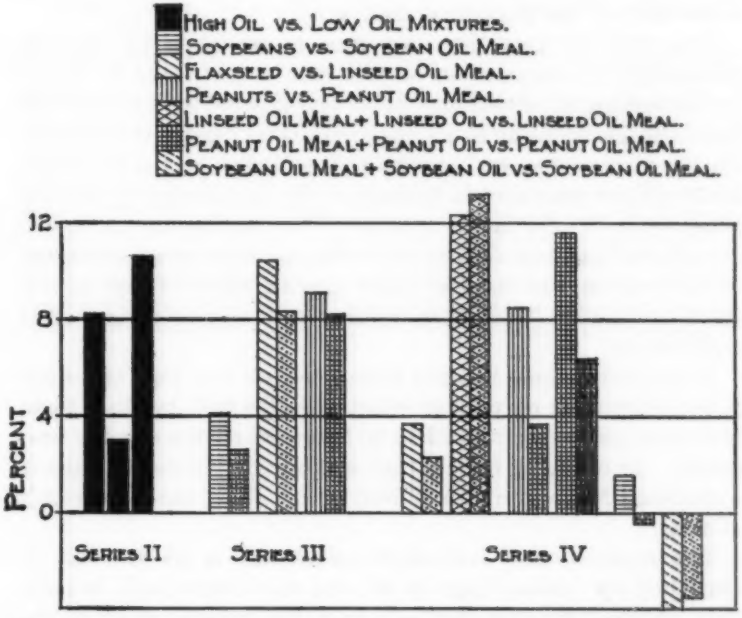

Fig. 1. Graphic Summary of Results Obtained in the Comparisons of "High Oil" with "Low Oil" Rations

The heights of the columns represent the percentage increases obtained. The columns in Series II and the left sides of the double columns in Series III and IV, represent the percentage increases in test, whereas the dotted right sides of the double columns represent the percentage increases in yields of fat.

parison of soybeans with soybean oil meal showed little effect, but there were distinct increases in the test due to flaxseed feeding compared with linseed oil meal and to feeding peanuts in

contrast to peanut oil meal, although these were of less magnitude than before and in one or two cases were small or negative. The feeding of linseed oil and peanut oil as components of rations containing linseed oil meal and peanut oil meal, respectively, caused increases of about 12 per cent in the test of the milk as compared to iso-dynamic oil meal rations. Fat yields were increased about 13 per cent by the linseed oil feeding and about 6 per cent by the peanut oil feeding.

It is believed that these experiments establish that (*a*) the percentage fat composition of milk can be materially increased by the feeding of rations high in oil as compared with rations low in oil but furnishing an equal amount of total digestible nutrients; (*b*) the increase can be induced by the feeding of ground whole seeds such as peanuts and flaxseed, or by the oils extracted from these seeds; (*c*) the factor responsible for the increase in test is the oil itself and not a stimulation due to a large excess of energy in the rations, nor to a so-called specific effect of any of the feeds used other than that which may be closely associated with the oil.

It was noted that changing from a low oil to a high oil ration is not followed at once by an effect upon the test, but that there is a latent period of from 12 to 36 hours before the effect is produced. In changing from a high oil to a low oil ration there is a residual effect of the high oil feed lasting about the same length of time.

The length of time over which an increase in the test can be sustained by rations high in oil was not determined, but the data obtained indicate that as a rule, the greatest effect is obtained within the first two to three days and that the test then tends to decline.

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APPENDIX

Note: In the charts which follow, economy of space has been sought by superimposing one column upon another. It is assumed that the bases of all columns rest upon the horizontal line at the zero point of the scale. The amounts of digestible protein and total digestible nutrients may thus be read directly from the scale at the left. The amounts of digestible fat may likewise be read in the same manner, but in order to make these visible in the charts, the actual amounts consumed were multiplied by ten in figures 5 to 7, and by six in the remainder.

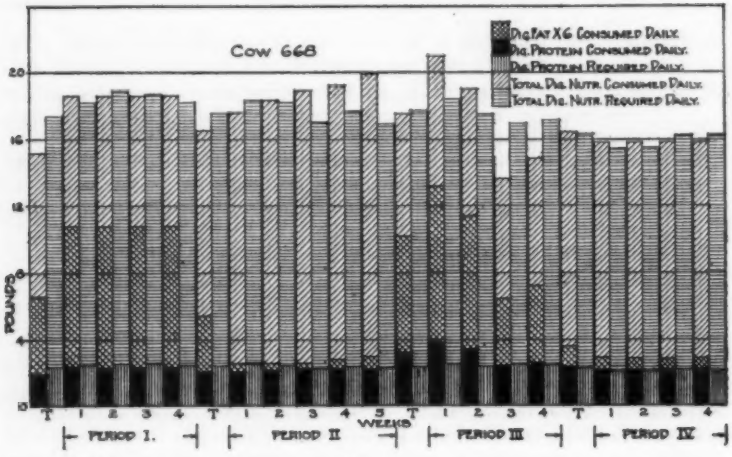

FIG. 2. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COW 668 DURING SERIES I

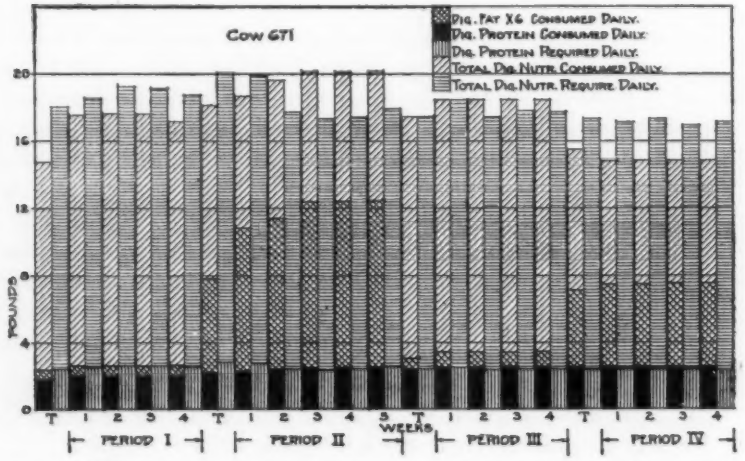

FIG. 3. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COW 671 DURING SERIES I

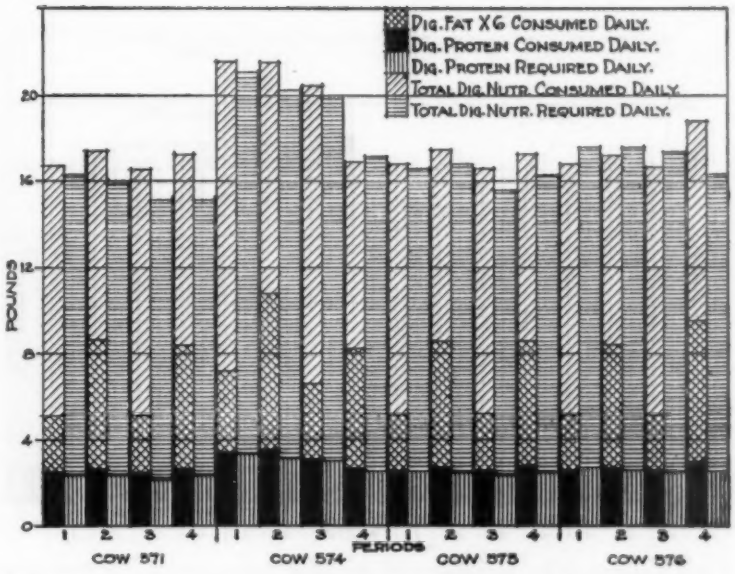

FIG. 4. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COWS DURING SERIES II

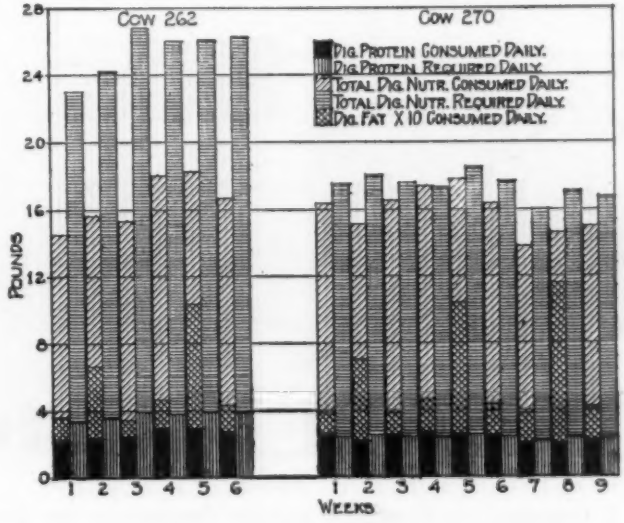

FIG. 5. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COWS 262 AND 270 DURING SERIES III

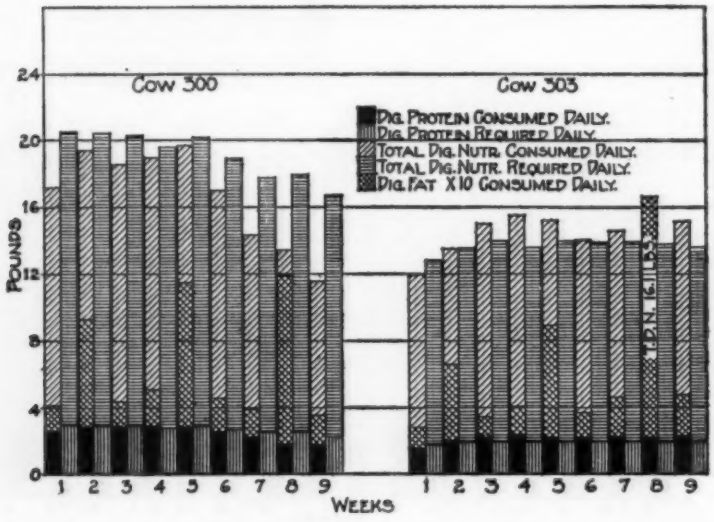

FIG. 6. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COWS 300 AND 303 DURING SERIES III

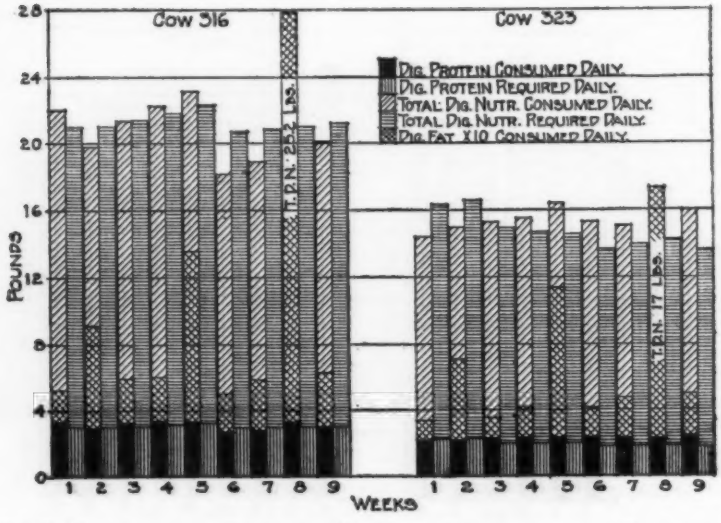

FIG. 7. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COWS 316 AND 323 DURING SERIES III

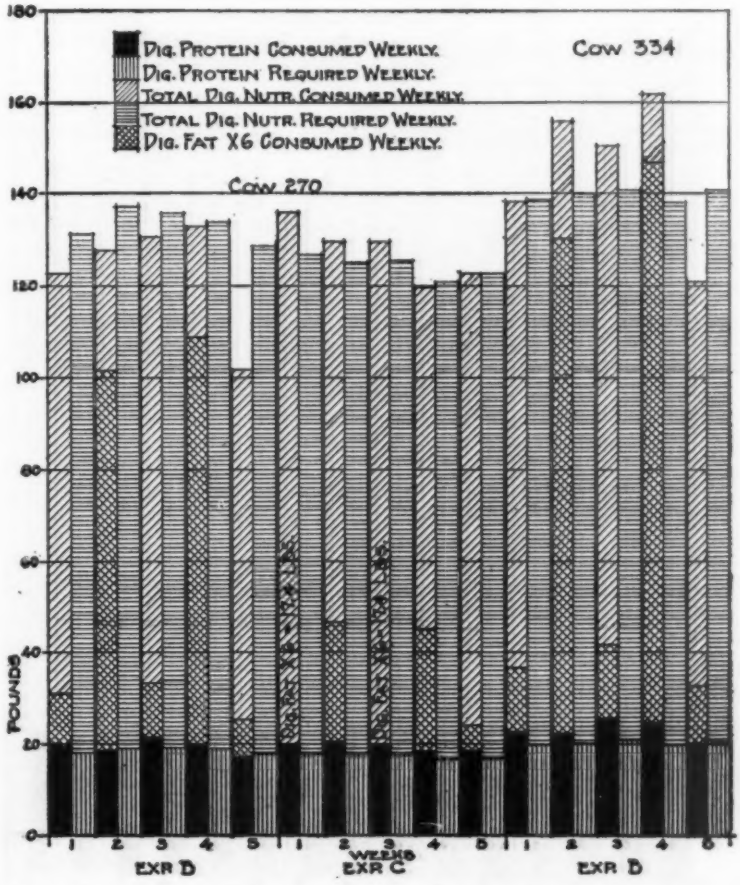

FIG. 8. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COWS 270 AND 334 DURING SERIES IV

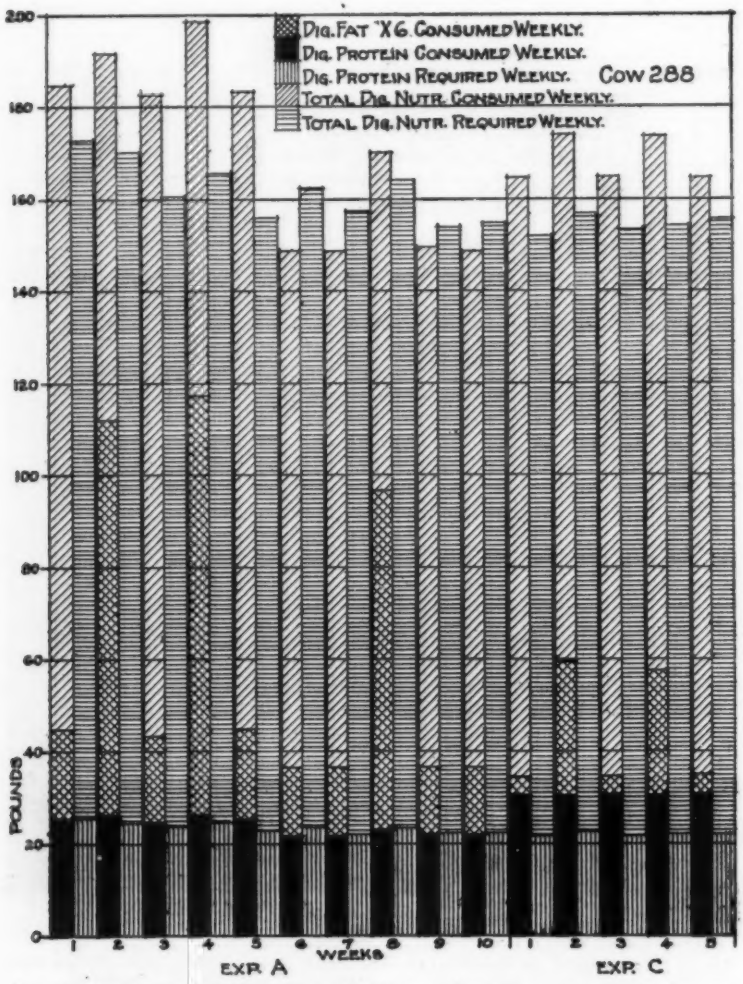

FIG. 9. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COW 288 DURING SERIES IV

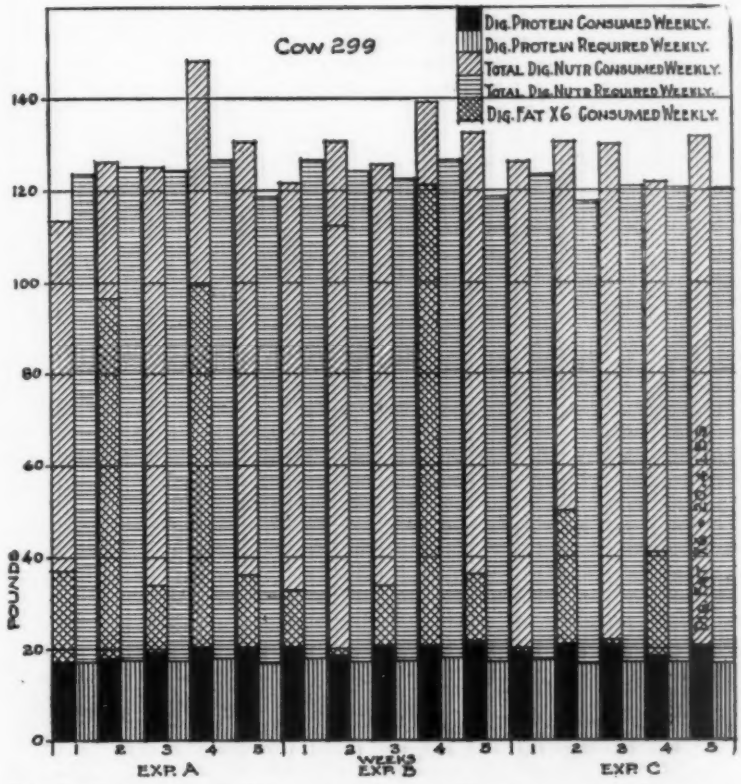

FIG. 10. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COW 299 DURING SERIES IV

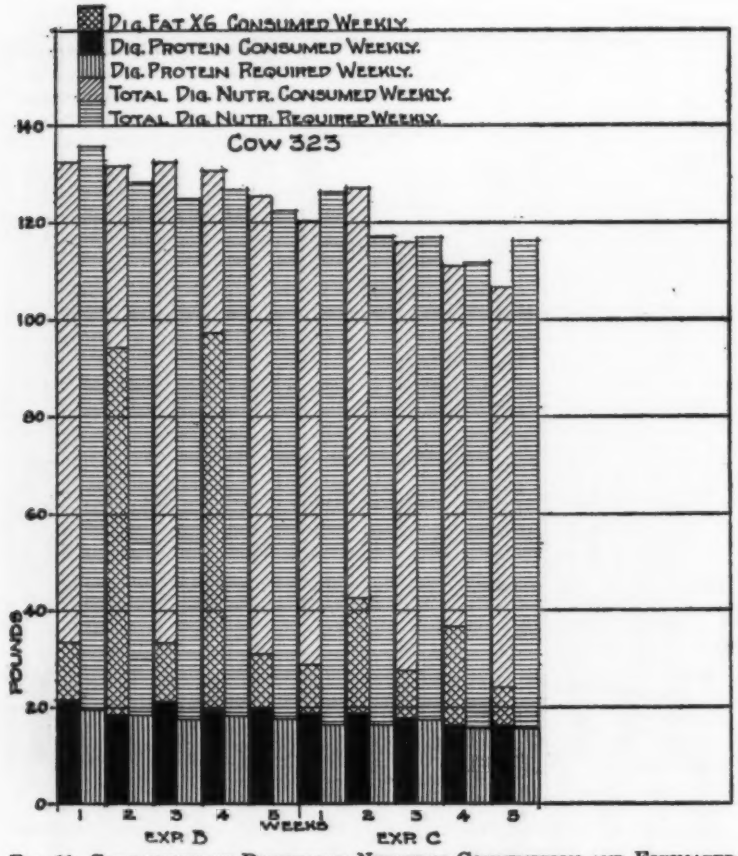

FIG. 11. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COW 323 DURING SERIES IV

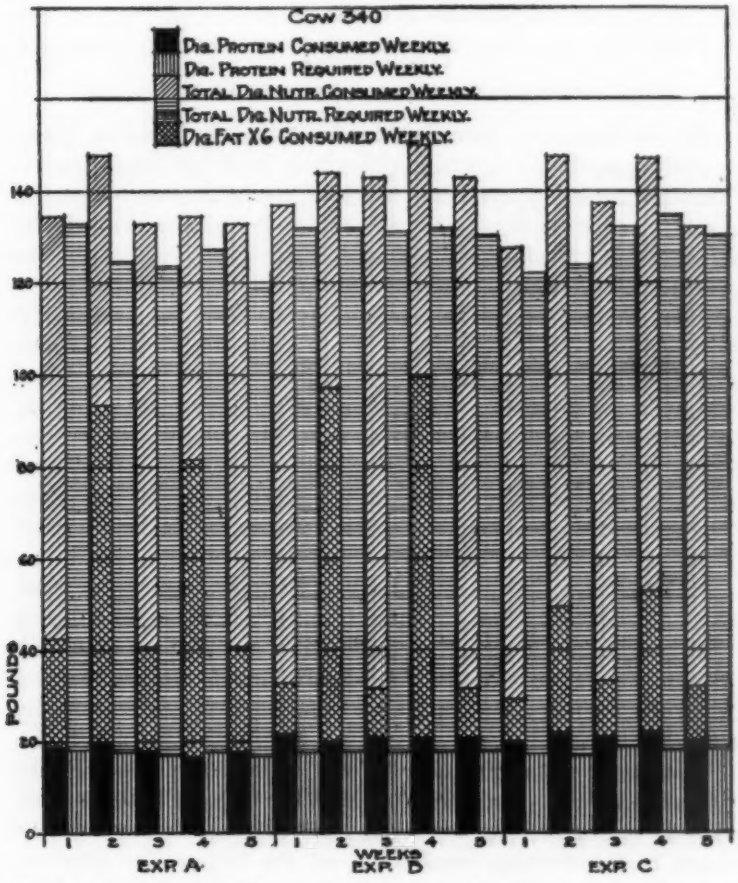

FIG. 12. COMPARISON OF DIGESTIBLE NUTRIENT CONSUMPTION AND ESTIMATED REQUIREMENTS OF COW 340 DURING SERIES IV

SHRINKAGE OF PRINT BUTTER*

E. S. GUTHRIE

Dairy Industry Department, Cornell University, Ithaca, New York

One of the most annoying considerations in handling butter is shrinkage, which makes it difficult to maintain legal weights. This is particularly true when it is held in cold storage for several months and when there has been sufficient time for evaporation of moisture. Shrinkage of butter is largely loss of water, and the decrease in moisture content is due mostly to evaporation.

This phenomenon is accelerated when the moisture is not thoroughly mixed with the fat. It is decreased when these two components of butter are so thoroughly worked together that the moisture droplets are small and thoroughly encased in fat. It is, therefore, apparent that the evaporation of moisture from butter, which may result in a shrinkage in weight below the legal limit, can be one of the butter dealer's most perplexing problems. The amount of working that butter receives probably has more to do with this loss of moisture than has any other factor.

In 1921 the author made a study of the effect of different types of tin-foil wrappers for one-pound butter prints on the keeping quality and the shrinkage of butter. It was easy at that time to obtain additional data on the loss in weight by evaporation when the butter was worked only sufficiently to prevent mottles and on additional working to better incorporate the moisture.

The butter was the regular make of sweet-cream goods in the Cornell University creamery. The Victor churn with one set of rolls was used. At the usual time of 50 revolutions of the churn in the working process, all of the butter was removed from the churn with the exception of one 90-pound Friday boxful. This butter was worked 18 additional revolutions. Seventy-five prints of the butter worked 50 revolutions and seventy-six prints of the butter worked 68 revolutions were put in the experiment.

* Received for publication April 30, 1926.

The prints were weighed on a Torsion butter scale which is graduated to read in thirty-seconds of an ounce. It was weighed on October 27, November 19, November 26, December 10, December 24, January 7, January 20, February 11, March 2,

TABLE 1

Effect of working on shrinkage of butter

SAMPLE	OCTOBER 28 TO NOVEMBER 5	NOVEMBER 12	NOVEMBER 19	NOVEMBER 26	DECEMBER 10	DECEMBER 24	JANUARY 7	JANUARY 20	FEBRUARY 11	MARCH 2	APRIL 10	NUMBER OF PRINTS	TOTAL LOSS*	AVERAGE LOSS* PER PRINT
Division 1. Worked 50 revolutions of Victor churn														
A	−23*	+1	−3	−5	0	−4	−8	−5	−2	0	−27	12	76	6.33
B	−23	−8	−3	0	−8	−8	−9	−4	−10	0	−8	13	81	6.23
C	−12	−1	−23	−2	−4	−5	−18	+2	−2	−21	−1	13	87	6.69
D	−13	−6	−2	−8	−4	−9	−15	−3	−8	0	−9	13	77	5.92
E	−8	−12	+3	−20	−8	−6	−16	0	−10	0	−20	12	97	8.08
F	−14	+37	−13	−7	−16	−1	−14	−2	−19	−4	−19	12	72	6.00
Total...	−93	+11	−41	−42	−40	−33	−80	−12	−51	−25	−84	75	490	6.54
Division 2. Worked 68 revolutions of Victor churn														
A′	−7	−8	+19	−16	−6	−5	−8	−7	+1	−13	−7	13	57	4.38
B′	+2	+8	−5	−17	0	−9	+5	−10	−19	−5	−3	13	53	4.08
C′	−5	+3	−2	−3	−2	−18	+10	+11	−11	−1	−16	12	34	2.83
D′	−12	−14	+6	+2	−1	−2	−3	−21	−8	−2	−3	13	58	4.46
E′	+6	−11	−10	−24	+1	−9	−9	−4	+29	−21	−14	13	66	5.07
F′	−3	−1	+1	−16	−8	+2	−12	−6	−2	−15	+2	12	58	4.83
Total...	−19	−23	+9	−74	−16	−41	−17	−37	−10	−57	−41	76	326	4.29

* Loss in moisture in thirty-seconds of an ounce.

and April 10. The total number of days in which this butter was held was 155.

The shrinkage from one weighing to another on the total weight of the 12 or 13 pound prints is shown in table 1. For illustration, sample A lost $\frac{23}{32}$ of an ounce from October 28 to November 5, and increased $\frac{1}{32}$ in the next period. Then it lost $\frac{3}{32}$ from November 19 to November 26. The total shrink-

age is given in this table, as well as the average for each pound print. The computation included each weighing.

TABLE 2

Effect of working on shrinkage of butter

SAMPLE	CHURNING 1		CHURNING 2		CHURNING 3		CHURNING 4	
	Age 234 days. First weight after storage	Age 261 days. Second weight after storage	Age 228 days. First weight after storage	Age 255 days. Second weight after storage	Age 220 days. First weight after storage	Age 247 days. Second weight after storage	Age 219 days. First weight after storage	Age 246 days. Second weight after storage
Division 1. Worked sufficiently to give uniform color								
A	−9*	−10	−23	−19	−15	−16	−22	−23
B	−14	−15	−20	−17	−8	−9	−11	−11
C	−12	−17	−29	−25	−20	−22	−24	−25
D	−15	−16	−23	−21	−17	−19	−12	−12
E	−20	−20	−28	−28	−21	−23	−27	−28
F	−15	−15	−26	−25	−20	−20	−20	−21
G	−8	−8	−19	−17	−16	−17	−20	−20
H	−9	−8	−26	−25	−15	−15	−17	−18
Average..	−12.75	−13.62	−24.25	−22.12	−16.50	−17.62	−19.12	−19.75
Division 2. Worked 10 additional revolutions of Victor churn								
I	−1	−1	−11	−11	−3	−3	0	−1
J	−1	0	−6	−6	−5	−5	−2	−2
K	−2	−1	−7	−7	+2	−1	−2	−2
L	−2	−1	−6	−6	−7	−5	−3	−3
M	−1	0	−10	−11	−4	−3	−3	−3
N	−0	+1	−12	−12	−5	−5	−3	−4
O	−1	0	−6	−6	−2	−2	−7	−6
P	−1	−2	−7	−7	−4	−3	−6	−6
Average..	−1.12	−0.50	−8.12	−8.25	−3.50	−3.37	−3.25	−3.37

* Loss in moisture in thirty-seconds of an ounce.

A and A′ were wrapped in the same kind of wrapping paper. Likewise, B and B′ and the other pairs were wrapped in the same types of paper. It is noticeable that in every pair the

butter which was worked 68 revolutions of the churn did not shrink as much as did that which was worked only 50 revolutions. Some of the variations were probably caused by the moisture leaking from one print to another, for it should be remembered that this butter was packed in 50-pound boxes which contained two tiers of 25 pounds each. The prints were not always replaced in a certain position in the box after weighing. Sometimes they were in the lower tier and again they were in the upper layer. This may account for occasional plus weights. The rate of shrinkage per day from weighing to weighing throughout the whole period seemed to be approximately the same. This indicates that the butter was still a long way from constant weight, or when no more evaporation would take place.

The average loss in the 155 days at 45° to 50°F. was $\frac{6.54}{33}$ of an ounce in division 1, and $\frac{4.29}{33}$ of an ounce in division 2. The difference of $\frac{2.25}{33}$ of an ounce to each one-pound amounts to 4.4 pounds on a 1000-pound churning. When the price of butter is 45 cents a pound, the value of this difference is $1.98.

More recent studies of shrinkage by the author indicate greater loss of moisture than is shown in table 1. The further research, which is reported in table 2, was made of the regular sweet-cream butter which is manufactured in the Cornell University creamery. It was held at 0 to +5°F. for about eight months, and was churned in a Victor churn which has two sets of rolls. When the butter was worked sufficiently to distribute the salt evenly, thus insuring a uniform color, the samples of division 1 were taken. Then the butter was worked 10 more revolutions of the churn and the samples of division 2 were obtained.

The average loss of moisture to each pound print of butter in division 1 was $\frac{18.15}{32}$ of an ounce, when first weighed after storage. When it was held 27 days longer and weighed the second time after storage the loss had increased to $\frac{18.28}{32}$ of an ounce, or 3.56 per cent of a pound. In division 2, the shrinkage was much less, for the first weight after storage showed only $\frac{3.25}{32}$ of an ounce, and it was $\frac{3.87}{32}$ of an ounce, or 0.75 per cent, 27 days later when it was weighed the second time after storage.

The shrinkage of 3.56 per cent of division 1 amounts to 35.6 pounds on a 1000-pound churning. The loss in division 2 of 0.75 per cent is 7.5 pounds in a churning of similar size. The difference is 28.1 pounds of butter, which at 45 cents a pound has a value of $12.65.

This limited study indicates that the shrinkage of butter by evaporation and leakage can be largely controlled by thoroughly incorporating the moisture in the fat in the working process.

A DEFECT OF PIMENTO CHEESE*

DONALD H. WARREN[1]

Research Laboratories of the Bureau of Dairying, United States Department of Agriculture, Washington, D. C.

INTRODUCTION

Pimento cheese is a variety of soft unripened cheese of the Neufchatel type which combines high moisture content with a high acidity. The quality of this type of cheese is largely dependent upon a clean, mildly acid flavor.

The work of this investigation was undertaken to determine the factors responsible for the sharply acid and bitter flavor which develops on standing and renders the cheese unsalable. Spore forming anaerobic bacilli like the causal organism of this defect have been studied by Esty (1), Hall (2), Reddish (3), Speakman (4), and others.

All experimental cheese was made in the laboratory where the entire manufacturing process could be controlled and uniform methods employed. The milk was received daily from the Bureau of Dairying farm and was pasteurized before being used. In making the cheese the usual factory procedure was followed throughout.

EXPERIMENTAL

In sampling the cheese for bacteriological analysis every precaution was taken to prevent contamination. The sample was removed from the center of the jar; and after weighing on a sterile paper, one gram was transferred to a mortar containing washed, ignited, sterile sand. Samples of cheese and the culture of Streptococcus lactis used in its manufacture were plated on litmus-lactose agar, litmus-dextrose agar, infusion agar, the milk

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[1] The assistance of Dr. W. C. Frazier throughout the investigation is greatly appreciated, as is also that of Mr. K. J. Matheson, at whose suggestion the study was undertaken.

powder agar of Ayers and Mudge (5), and a 10 per cent skimmed-milk agar. The last two media proved the most satisfactory, for they produced colonies that were larger and the acid-forming types could be more readily differentiated. Dilutions of 1:1,000,000 and 1:5,000,000 gave the most satisfactory plates. All plates were made in duplicate and incubated for three days at 26°C. Later anaerobic methods were tried.

The total titrable acidity and the volatile acidity of the cheese were determined. The total titrable acidity was determined by titration with an N/10 solution of sodium hydroxide, using phenolphthalein as an indicator. An aqueous extract of the cheese did not prove satisfactory, because, as Barthel (6) has demonstrated, the acids arising from the fermentation of the milk sugar are mainly in combination with the lime of the phosphate and the paracasein. The determinations were made in duplicate, and the figures represent an average. Cheese finely ground in a mortar was used for these titrations.

The volatile fatty acids present in the cheese were determined quantitatively by the steam distillation method of Ducleaux (7). A 100-gram sample was weighed out and placed in a large mortar with 100 cc. of distilled water. The mass was thoroughly ground with a pestle and an additional 200 cc. of distilled water placed in the receiving flask, after rinsing all particles from the mortar. Five grams of granulated zinc and 2 cc. of concentrated sulphuric acid were added to the contents of the boiling flask to bring over the volatile acids. The hydrogen liberated by the combining of the zinc and sulphuric acid kept the contents from violent agitation during the distillation process. Thirty cubic centimeters of N/10 sodium hydroxide were placed in the receiving flask and the distillation continued until 1000 cc. of the distillate was secured. This volume was titrated with N/10 HCl, using phenolphthalein as an indicator.

Streptococcus lactis was eliminated as a possible cause of the defect since data secured from three cultures over the usual six-day period showed that little change in the amounts of volatile acid took place, while no defect in flavor could be noted. This is in agreement with the results of Hammer (8), Evans (9), Leichmann (10), and Orla Jensen (11).

Bacteriological examinations of a number of samples of canned pimentoes showed that these were sterile. Both the plate method and the anaerobic technique were employed. Since the defect could be produced in cheese without the addition of pimentoes, these were eliminated as the causative factor of the sharply acid and bitter flavor.

Six lots of cheese were made in the usual manner, and daily analyses were run over a six-day period, which represents the time during which the marked sharpness and bitterness usually appeared. A study of the plates showed that 98 per cent of the organisms present during the development of the defect were acid formers of the Streptococcus lactis type. A marked increase in volatile acidity took place, ranging from 14.7 to 34.9 per cent with an average increase of 29.1 per cent. The sharply acid flavor developed two or three days before the bitterness occurred. It was apparent from these experiments that the causative organism could not be demonstrated by aerobic methods; therefore, anaerobic methods were tried.

A 1-gram shample of cheese with the sharpness and bitter flavor well developed was shaken well in 100 cc. of sterile water. Four tubes containing approximately 10 cc. each of dextrose-agar were melted in a steamer and quickly cooled to 40°C. One cubic centimeter or $\frac{1}{100}$ gram of cheese from the above 1:100 dilution was added to the first tube of dextrose-agar. In a similar manner 1 cc. was transferred from tube 1 to tube 2. This serial dilution was carried through four tubes. The work was done in duplicate, and the surface of the inoculated media of the first set of tubes was covered with melted vaseline. The second set was drawn into sections of sterile glass tubing sixteen inches long. These were filled to within an inch of each end and allowed to solidify after which all were incubated at 37.5°C. from twenty-four to forty-eight hours.

Examination at the end of twenty-four hours showed the characteristic anaerobic gas production, both in the test-tube cultures and in those in the glass tubing. This occurred through the third tube of the series which contained approximately $\frac{1}{1{,}000{,}000}$ gram of cheese. Growth was not apparent in the higher

TABLE 1

Cheese made from milk sterilized in the autoclave

DAY	TOTAL TITRATABLE ACIDITY	TOTAL VOLATILE ACIDITY	ROUX TUBES DILUTION SHOWING GAS	PLATE COUNT PER GRAM	MICROSCOPIC	FLAVOR
Lot 1. Control cheese inoculated with 0.5 per cent of Streptococcus lactis alone						
	per cent	*cc. N/10 NaOH*	*gram*			
First	1.60	17.00	Negative	1,520,000,000	Cocci	Clean, mildly acid
Second	1.65	17.20	Negative	846,500,000	Cocci	Clean, mildly acid
Third	1.65	17.25	Negative	611,500,000	Cocci	Clean, mildly acid
Fourth	1.75	17.50	Negative	329,500,000	Cocci	Clean, mildly acid
Fifth	1.75	17.60	Negative	342,500,000	Cocci	Clean, mildly acid
Sixth	1.78	17.85	Negative	386,500,000		Clean, mildly acid
Lot 2. Cheese inoculated with 0.5 per cent Streptococcus lactis and anaerobic culture of "shocked "spores						
First	1.78	17.20	Negative	218,500,000	Cocci	Slightly sour
Second	1.85	17.40	1/10,000	247,500,000	Cocci	Increased sourness
Third	1.88	17.65	1/10,000	184,000,000	Cocci few medium rods	Sharply sour
Fourth	1.90	19.45	1/10,000	135,500,000	Cocci few medium rods	Very sharply sour marked slight bitterness
Fifth	1.95	20.10	1/1,000,000	71,000,000	Cocci rods predominate	Very sharply sour, very bitter
Sixth	1.97	20.50	1/1,000,000	52,500,000	Cocci	Very sharply sour, very bitter

Lot 3. Cheese inoculated with Anaerobic Culture of Shocked Spores Alone

First.....	1.65	17.50	1/10,000	18,000	Medium sized rods	Very sour
Second.....	1.65	17.40	1/10,000	5,200	Medium-sized rods	Increased sourness
Third.....	1.72	17.80	1/10,000	27,000	Medium-sized rods predominating few small cocci	Extreme acid flavor
Fourth.....	1.80	18.90	1/1,000,000	3/800	Medium-sized rods	Slight bitterness
Fifth.....	1.82	19.20	1/1,000,000		Medium-sized rods	Very bitter
Sixth.....	1.84	19.20	1/1,000,000	7,000	Medium-sized rods	Very sharp and bitter

dilutions after 48 hours incubation. The presence of this anaerobe was also demonstrated in three market samples having the characteristic defect well developed. The increase in the volatile acidity coincident with the marked changes in flavor occurring in these market samples was in accordance with the results secured on the six samples of the laboratory-made cheese. This was considered sufficient proof that the cause of the defect occurring in the laboratory cheese was the same as that of the commercial product. The anaerobe was isolated and found to be similar to Clostridium butyricum (B. amylobacter) in its characteristics.

The sharp bitterness which occurred in the cheese with large numbers of the spore-forming anaerobic bacilli present made it seem likely that the cause of the defect was this anaerobe. If the anaerobic culture isolated from the laboratory-made cheese and the commercial samples was the causative factor in the production of the sharp bitter flavor in these cheeses, it should be possible to reproduce these changes in specially controlled cheese by inoculation with this anaerobic culture.

Since investigators working with the butyric type of fermentation caused by B. amylobacter have shown that a more vigorous fermentation occurred when the spores of the organism had been "shocked" or subjected to high temperatures, this process was also tried in this experiment.

The milk used for the experiments in table 1 was sterilized in the autoclave and divided into three lots. Each of the three lots was inoculated with 0.5 per cent of a twenty-four-hour culture of Streptococcus lactis. To lot 1, which was the control, nothing was added. Lot 2 was inoculated with 0.5 cc. of the "shocked" spores of anaerobic organism. This culture of "shocked" spores was prepared by heating old cultures of the anaerobe to 85°C. for fifteen minutes. Lot 3 was inoculated with 0.5 cc. of the unheated (normal) spore culture of the anaerobe. Every precaution was taken to avoid contamination throughout the manufacture of the cheese. A microscopic examination of each lot was made daily using the Breed technique.

The data in lot 1, which is the control cheese, showed that the

total titrable acidity and volatile acidity were only slightly increased. The flavor remained clean and mildly acid throughout the entire period. The per cent of increase in volatile acidity was 4.7. This, together with the negative Roux tubes, the plates, and the microscopic examination, is believed to be ample evidence that the control cheese had not become contaminated.

The data in lot 2, which contains the shocked spores of the anaerobe, showed that a marked increase in volatile acidity took place. The increase was 16 per cent as compared with an increase of only 8.8 per cent in lot 3, where the unheated spore culture was used. The marked increase in volatile acidity accompanied by a more rapid development of the bitter flavor in the cheese having both Streptococcus lactis and the spore culture of the anaerobe is considered strong evidence of an associative action of these two organisms in the development of the defect. The bitterness was noticeable early on the fourth day, and the cheese became very bitter by the end of the fifth day. The anaerobe was demonstrated in the $\frac{1}{1,000,000}$ gram dilution on the fifth day. The defect described above appeared more rapidly and was more pronounced in the experimental cheese having the "shocked" spore culture of the anaerobe.

The anaerobic organism used in these experiments is a gram positive motile bacillus of medium size. Colonies in deep dextrose-agar tubes are compact with a very definite darker center. Litmus milk is rapidly reduced and coagulated, followed by abundant gas production. The curd is fragmented and the butyric-acid odor is very noticeable. Indol is not formed. Nitrates are reduced to nitrites. The organism appears similar to Clostridium butyricum (Bacillus amylobacter) according to Bergey's classification.

SUMMARY

A brief summary of the results of this work is given below:

1. Streptococcus lactis was proved not responsible for the sharply acid and bitter flavor.
2. The pimentos were not responsible for the defect.
3. Aerobic methods failed to disclose the causal organism.

4. Nine samples of sharply acid and bitter cheese were found to contain large numbers of spore-forming, gram positive, motile anaerobic bacillus which resembles Clostridium butyricum.

5. The increase in sharply acid and bitter flavor was always accompanied by a corresponding increase in volatile acidity.

6. The characteristic defect was produced experimentally by the use of a pure culture of the anaerobic bacillus.

7. "Shocked" spores of the anaerobic bacillus produced a more active fermentation than the unheated spores.

8. Pasteurization of raw milk is equivalent to this method of shocking the spores.

9. The associative action of the Streptococcus lactis and the anaerobic bacillus proved more effective than the anaerobe alone.

CONCLUSION

The sharply acid and bitter flavor, commonly found in old pimento cheese of the Neufchatel type, is due to the action of an organism resembling Clostridium butyricum, which may act in association with the Streptococcus lactis of the starter.

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ELIMINATING THE TOXICITY OF COTTONSEED MEAL*

WILLIS D. GALLUP

Department of Agricultural Chemical Research, Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma

INTRODUCTION

Studies to determine the cause of deleterious effect arising from the feeding of cottonseed meal to certain types of live-stock and other animals, have been carried out to such an extent and such evidence brought to light on the subject, that we feel quite safe in assuming the trouble to lie in the toxic properties of a substance isolated from the cotton seed and called gossypol (1, 2). Such an assumption is not without criticism however, and it does not necessarily follow that wherever injurious effects are encountered with the use of cottonseed meal as a feed, the only cause is due to gossypol. Yet, the principal cause of the injury, when balanced rations are being used, is no doubt due to this substance.

Other substances which have been suggested as productive of the symptoms of toxemia and malnutrition have been investigated and the results summarized by Macy in this JOURNAL (3). Among such substances are the nitrogenous compounds Cholin and Betain, the high protein content, the fiber, parasitic organisms, decomposition products, oil content, and compounds of phosphorus. Other investigators have suggested that the trouble is due to a deficient diet (4, 5, 6, 7). Most of our later evidence, however, is not in favor of these theories, and the results of recent investigations carried on to a large extent by Southern Experiment Stations tend to prove that gossypol is the main cause of the trouble. Unpublished results at this station also point in that direction.

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[1] These animals were obtained without charge from V. G. Heller.

We are particularly interested in cottonseed meal as a foodstuff for several reasons and feel that too much stress cannot be laid on the needs for investigation of its use in feeding different kinds of farm animals. Henry and Morrison (8) state, "Since the meal is a heavy, highly nitrogenous feed, and is poisonous to fattening cattle when fed in excess, the determination of the allowance to be fed for the best results is of great importance." These authors go on to say, "It is to be hoped that in time methods may be developed of treating cottonseed meal so that it will be safe for all classes of animals." Much of the work at this station has been conducted along this last line of investigation and we have met with very favorable results.

Not only is cottonseed meal highly nitrogenous but it contains protein of such excellent quality that when the meal furnishes the sole source of protein in a diet for albino rats, growth and reproduction are normal (5). This is a fact not true for many feeds. The work of Nevens (9, 10) on the amino acid content and nutritional value of cotton seed protein has contributed much to our knowledge of this important factor. Nevens found that when cottonseed meal furnished the sole source of protein in a ration containing 10 per cent crude protein, the utilization of the protein for the growth of albino rats was 66 per cent. In a similar way he found the utilization of the protein of alfalfa to be 62 per cent and that of corn 49 per cent. His investigation was carried out by first studying the endogenous metabolism of the experimental animals during a metabolism period in which a nitrogen-free ration was fed and then followed by six such metabolism periods in which the proteins of these feeds were compared.

The digestibility of the protein from cotton seed as found by other investigators (11, 12, 13, 14) is not in close agreement but probably lies between 80 and 85 per cent. Rather (13) finds this value to the 78.6 per cent which is about the same as that for the protein of legumes, but less than the value as found for cereals and meat. Since the isolated cottonseed globulin, even when fed in small quantities (15) has been shown to be able to support normal growth, Jones and Waterman (14) believe that the isolated protein is more digestible than the protein in combination with

other substances in cotton seed and conclude that the lower digestibility is due in part at least to the presence of gossypol. The digestibility of cottonseed meal in which the gossypol has been removed or destroyed would be well worth studying.

As a result of the foregoing investigations together with many practical experiments on the feeding value of cottonseed meal it seems desirable that we learn more concerning its use in large quantities without deleterious results to the animals fed. The most important facts with which the feeder is concerned are, that the meal contains a high per cent of protein which is of excellent quality and quite digestible, that the price of the meal is comparatively low, and that the manurial value is exceptionally high. Its toxicity, however, is a drawback to its extensive use.

Macy (3) (1921) states, "Many attempts have been made to render cottonseed meal non-toxic, but none have been successful for all species alike." Among the attempts are those of Edgerton and Morris (16) who subjected the meal to various treatments such as digestion in hot water, 95 per cent alcohol, and ether; fermentation, heating with hot air, over oil, and autoclaving the meal in both a dry and wet condition. All their experimental animals (guinea pigs) either lost weight or died. Withers and Ray (17) were able to reduce the toxicity of the meal by boiling it with alcoholic sodium hydroxide for two hours. Later, iron salts were suggested as an antidote and Withers and Carruth (18) found that when iron and copper salts were fed with the meal, pigs made better gains. Osborne and Mendel (15) who fed cotton seeds previously steamed for various lengths of time and then dried were unable to draw any definite conclusions to the benefit of such treatment. The latest work has been done by Dowell and Menaul (19) who show that autoclaving the wet meal at 15 pounds pressure for twenty minutes destroys the poison peculiar to it and makes a safe feed for young pigs.

Recent investigations have been carried out principally along two lines. One of these is based on the theory that the injury is due to a deficient diet, while the other has for its foundation, the isolation of the toxic gossypol. Many feeders believe that the conditions under which animals are kept when being fed on cot-

tonseed meal have much to do with the results of such feeding and when the animals are on pasture they are able to consume much larger amounts of meal without bad effects. Also that silage makes an exceptionally good supplement. These things might well be true, and it is barely possible that the supplement used may be of such a nature that it inhibits the effect of gossypol or possibly combines with it to form an insoluble compound or destroys it altogether. Such possibilities must be kept in mind when considering the real cause of cotton seed injury. It is more probable that certain supplements have a specific effect on the gossypol than that they make up for a deficiency which would otherwise result in injury to the growth of the animal. Iron salts have been proven to be beneficial to the health of pigs receiving cottonseed meal and since we know that gossypol forms an insoluble compound with iron we might well believe that their corrective effect is due in part to their combination with gossypol in the meal. What effect silage and the compounds contained therein might have upon gossypol is well worth investigating. The value of pasture and source of roughage fed with the meal is subject to diverse opinions.

The dairy industry is particularly interested in cottonseed meal since it constitutes one of the principal sources of protein and any condition which may increase the food value of this substance will be of direct interest to it. Moore (20) reports that for cows as much as 5 pounds per head daily is injurious if fed for any length of time and that it causes difficult breathing and inflammation of the udder, while for calves it has been reported that as much as ¼ to ½ pound per head daily is fatal (21). Calves and swine are especially susceptible to the effect of cottonseed meal feeding while sheep, as found by Dowell and Menaul (19) show no signs of ill effects even after being fed 1 pound of the meal daily for a period of ninety days. The dairy herd at this station is receiving a ration which contains about 10 per cent cottonseed meal and when this amount is increased, silage is usually fed as a supplement.

Recent reports at the North Carolina Station (22) show that calcium carbonate, butter fat, cod liver oil, yeast and other sup-

plements have a corrective effect when excessive quantities of cottonseed meal are fed to cows and heifers. Our own results, which were obtained by feeding albino rats a ration in which calcium carbonate, butter fat, and yeast supplemented the cotton seed, are not in harmony with theirs and are reported later in this paper. However, the methods of conducting the two experiments have probably been so diverse that the two results are hardly comparable.

The following investigations were undertaken for two principal reasons. First, to continue to work of Dowell and Menaul on the effect of autoclaving upon the toxicity of cottonseed meal and, second, to find out what effect certain supplements might have when large amounts of cotton seeds are fed. In doing this young pigs and albino rats were used as experimental animals. Although albino rats are not as susceptible to the effects of cottonseed meal as are guinea pigs, we were better equipped to care for rats and found that they responded quite readily to the effects of the cotton seed diets used. The results obtained by feeding young pigs are probably the same as might be expected in feeding calves similar rations and thus allow for somewhat general conclusions. According to Henry and Morrison (8) (1923), "No uniformly successful method of feeding cottonseed meal to swine has yet been found."

EXPERIMENT A

Twelve young pigs were divided into 4 lots of 3 each, using one lot for a control, and the other three for studying the effect of feeding commercial cold pressed cottonseed meal as it is brought on the market and the same meal autoclaved and steamed. All the pigs were innoculated against cholera and scrubbed with cresote to rid them of lice before the actual experiment began. One of the pigs in the pen receiving the autoclaved meal became sick shortly after the experiment began and was replaced by a pig from the control pen. The death of the animal, which occurred sometime later could not have been due to cotton seed injury since the feeding had only progressed a few days previous to the animal's sickness. The pigs were weighed at the

beginning and at the end of the experiment which lasted seventy-five days.

The autoclaved meal was prepared by dampening the commercial meal and autoclaving it at 20 pounds pressure for one hour. The time of autoclaving was somewhat longer than that used by other investigators (19) but by so prolonging the time the meal became more thoroughly cooked. The thoroughness of cooking was determined by the odor and color of the meal. The autoclaved meal when removed from the autoclave has a brown color throughout and a pleasant cooked odor. When this cooking treatment is not complete the center of the mass becomes only slightly brown and that portion of the meal retains its toxic properties. The steamed meal was prepared by passing steam directly into the meal contained in a large tin container with a false bottom. Holes were punched in the bottom of the container and it was packed with excelsior around the sides into a half barrel. A tap was fitted in the bottom of the barrel to allow the excess water formed by the condensed steam, to escape. The temperature was kept close to 100°C. Much of the steam condensed during the process which lasted for about one hour and the meal, although very wet when removed, was thoroughly cooked throughout.

The rations for the pigs receiving the cottonseed meal were made up of corn chop 3.3 pounds, cottonseed meal 1.2 pounds, and alfalfa. During the last month of feeding part of the corn chop was replaced with wheat bran but during the entire experiment the amount of cottonseed meal fed daily constituted approximately 1.3 per cent of the body weight of the animal. The amount of feed was thus increased proportionally as the pigs grew.

The control pen contained only two pigs receiving a ration composed of corn chops 3 pounds, tankage 0.5 pound, and alfalfa. These pigs did not appear as strong or eat as well as the others.

The pigs receiving the steamed and autoclaved meal were in the best condition throughout the experiment and always ate all of their feed. The pigs receiving the untreated meal ate sparingly, picking out the corn as much as possible from the meal, and often leaving considerable feed. By occasionally making a

slop of the uneaten residue with the next day's feed this condition was remedied.

The results of the experiment are shown in table 1 which gives the weights of the pigs in pounds, the form in which the cottonseed meal was fed to each lot, and the gains made.

TABLE 1

Growth of pigs on cottonseed meal rations

NUMBER OF PIG	ORIGINAL WEIGHT	FINAL WEIGHT	
		Lot I	
1	25	34	Untreated cottonseed meal
2	25	35	Total gain: 40 pounds
3	39	60	Gain per pig: 13 pounds
Total.....	89	129	
		Lot II	
1	33	53	Autoclaved cottonseed meal
2	23.5	51	Total gain: 66 pounds
3	37.5	56	Gain per pig: 22 pounds
Total.....	94	160	
		Lot III	
1	26.5	40	Steamed cottonseed meal
2	39	74	Total gain: 77 pounds
3	31.5	60	Gain per pig: 26 pounds
Total.....	97	174	
		Lot IV	
1	20	33	No cottonseed meal
2	18	35	Total gain: 30 pounds
Total.....	38	68	Gain per pig: 15 pounds

Although the pigs receiving the steamed cottonseed meal made the largest gains, none of the pigs increased in weight as rapidly as might be expected. This was probably due to the amount of bulk in the ration since cold pressed meal contains a large amount of crude fiber and does not therefore make as suitable a feed for

pigs as the hot pressed meal. The pigs which were in the poorest condition were used in the control pen in order that those receiving the cotton seed meal rations might be of about equal size and weight. The difference in the gains made by the control pigs and those receiving the cottonseed meal, therefore, cannot be used for comparing the two rations and further work would need to be done to obtain the feeding value of cooked cottonseed meal as compared to other concentrates.

EXPERIMENT B

In order to continue this work with animals which require a smaller amount of food and permit the use of purified forms, albino rats were used for experimental animals.[1] It had previously been found (15) that rats receiving in their diet cottonseed kernels which had been steamed from one to six hours grew better than those receiving the untreated kernels. However, even after steaming for one hour, the kernels proved to be still toxic and the rats thus fed lost weight. Furthermore, the rats which were fed kernels steamed for six hours made smaller gains than those fed the kernels steamed for two and four hours. It would seem from this that one hour of steaming was not sufficiently long to bring about the changes in the meal desirable to produce a safe feed, whereas six hours of steaming were too long. If the improvement of the kernels as produced by steaming is due to the destruction of the gossypol, then the above results would be expected to be more uniform.

Keeping in mind the possibility of feeding cottonseed products in a well supplemented diet without producing injurious effects, the rations shown in table 2 were made up and fed.

The cotton seeds which were obtained from the agronomy department were delinted with sulphuric acid and fed in the ration without removing the hulls. When used in rations II and III they were soaked about four hours previous to autoclaving. Such treatment softened the hulls and allowed the seeds to soak up considerable water such that when they were subsequently autoclaved, the steam was able to penetrate the kernel. Although the seeds were not autoclaved in such large quantities

as was the meal in previous work, yet this method of destroying the gossypol appeared to be more thorough and there was practically no loss of oil or other constituents. The cotton seeds used in ration IV were first extracted with ethyl ether which removes both the gossypol and oil. Commercial cottonseed oil was then added to the ration. Distilled water containing a trace of KI was supplied for drinking.

The result of this feeding which extended over a period of nine weeks is shown in table 3.

TABLE 2

Cotton seed rations for rats

	I	II	III	IV
	per cent	*per cent*	*per cent*	*per cent*
Wheat	50	50	50	50
Cottonseeds, untreated	35			
Cotton seeds, autoclaved at 20 pounds for one hour		35		
Cotton seeds, autoclaved at 20 pounds for two hours			35	
Cotton seeds, extracted				25
Butter	6	6	6	6
Agar agar	3	3	3	3
Yeast	5	5	5	5
$CaCO_3$	1	1	1	1
Cotton seed oil				10

All the animals receiving the uncooked cotton seeds were in poor conditions during the latter part of the experiment and died within a short time after the experiment ended. The other animals made about normal gains and grew to be healthy strong rats producing normal young. Death in all four cases was prolonged, the rats having made no consistent gains after the first three weeks but usually lost weight thereafter. Although acute cases of poisoning have been noted, it usually takes this slower form which makes recovery possible providing the symptoms are recognized and changes made in the diet.

From these results it is clear that autoclaving the cotton seeds as described is beneficial and allows them to be used in large

quantities in a ration which is supplemented with the necessary minerals and vitamines to produce normal growth. Autoclaving for two hours does not seem to have any advantage over the one hour treatment.

Since some investigators claim that the feeding of cottonseed meal produces no injurious effects upon albino rats and that the ether extracted meal has no advantage over the ordinary meal (5), it seemed desirable to find out if the steamed meal has any advantage over the ordinary meal. When working with the meal, other factors enter in which cannot be disregarded as is done when feeding the cotton seeds. The most important factor

TABLE 3

Growth of rats on cotton seed rations

FORM OF COTTONSEED	UNTREATED				AUTOCLAVED ONE HOUR				AUTOCLAVED TWO HOURS			EXTRACTED		
	Ration I				Ration II				Ration III			Ration IV		
	Rat 1 ♂	Rat 2 ♀	Rat 3 ♂	Rat 4 ♀	Rat 5 ♂	Rat 6 ♂	Rat 7 ♂	Rat 8 ♂	Rat 9 ♂	Rat 10 ♀	Rat 11 ♀	Rat 12 ♂	Rat 13 ♂	Rat 14 ♂
Original weight, grams	71	66	88	82	99	86	85	92	106	85	92	92	92	95
Final weight, grams	79	67	85	67	173	147	137	146	205	135	126	140	168	160
Gain or loss, grams	+8	+1	−3	−15	+74	+61	+52	+54	+99	+50	+34	+48	+76	+65

is the destruction of the gossypol or its conversion into a less soluble compound during the heating process previous to pressing out the oil. At the oil mill, the decorticated seeds before the oil is pressed out, are heated (for about thirty minutes at a temperature of about 100°C.), in steam-jacketed drums but are not subjected to the direct action of the steam. The oil is then pressed out of this hot mass, taking with it much of the gossypol. The remaining cake is broken up or ground and sold as "hot pressed" meal. During this preliminary heating some of the gossypol is undoubtedly destroyed but the amount must be very small and will vary with the process. A considerable amount of

the gossypol, however, may be altered such that it is less soluble in the oil and therefore retained in the meal. If this less soluble form is as toxic as the original form then we would expect to find the resulting meal almost as toxic as the seeds. Since this is not true, it is then supposed that the gossypol is rendered less toxic at the same time that it is converted into a less soluble compound and the completeness of this reaction will depend upon the conditions which prevail during the preparation of the meal. Some manufacturers heat the seeds for a much longer time than thirty minutes and in many cases the pressure of the steam surrounding the drums ranges from 30 pounds upward. Since the moisture content of the seeds is also quite variable and seems to influence the destruction of gossypol, methods by which the meal is produced will vary from one mill to another. Due to these conditions, no two samples of cottonseed meal as bought may be alike in their toxicity and it is due to this condition that cotton seed meal is sometimes reported as nontoxic to rats.

To determine the advantage of steaming the meal before feeding to rats, the rations given in table 4 were made up and fed to three groups of rats for a period of seven weeks.

The cottonseed meal used was purchased on the open market and when used in ration XVII was steamed as previously described in experiment A.

The rates of growth are shown in table 5.

As in the case where autoclaved cotton seeds were used, the steamed meal has a marked advantage over the ordinary meal and may be fed in much larger amounts. The use of only 25 per cent cottonseed meal did not appear to be toxic to the rats during the seven weeks of feeding and it is possible that, that amount of the meal can be fed to rats for a longer period of time without retarding their growth. However, by increasing this amount 10 per cent, slow growth and poor condition are noticeable in a short time.

Such a condition as this often arises in stock feeding when there is a certain limit beyond which the amount of cottonseed meal in the ration cannot be increased with safety. This difficulty

may be overcome without changing the other constituents of the ration by steaming the meal previous to its use.

Further proof of the advisability of such treatment and the increased nutritive value of the cotton seed is shown in the growth made by a group of rats fed solely on autoclaved cotton seeds and

TABLE 4

Cottonseed meal rations for rats

	XVII	XVIII	XIX
	per cent	per cent	per cent
Wheat	58	58	69
Cottonseed meal steamed one hour	35		
Cottonseed meal untreated		35	25
Butterfat	5	5	5
$CaCO_3$	1	1	1
NaCl	1	1	1

TABLE 5

Growth of rats on cottonseed meal rations

FORM OF COTTONSEED MEAL	STEAMED ONE HOUR				UNTREATED				UNTREATED	
	Ration XVII				Ration XVIII				Ration XIX	
	Rat 43 ♀	Rat 44 ♂	Rat 45 ♀	Rat 46 ♂	Rat 47 ♂	Rat 48 ♂	Rat 49 ♀	Rat 50 ♀	Rat 51 ♀	Rat 52 ♀
Original weight, grams	28	28	29	35	40	36	28	28	28	28
Final weight, grams	102	121	115	158	80	80	27*	31†	100	110
Gain or loss, grams	+74	+93	+86	+123	+40	+44	−1	+3	+72	+82

* Rat 49 ♀ died after five weeks feeding.

† Rat 50 ♀ died after three weeks feeding.

a 2 per cent salt mixture composed of equal parts of NaCl and $CaCO_3$. Their growth is shown in table 6.

Rats 29 ♂ and 30 ♂ both made small gains until the time of their death which occurred before the experiment ended. Rats 27 ♀ and 28 ♂ gained slowly during the first eleven weeks of feeding and then declined in weight until June 26 when 5 per cent butter fat was incorporated in the ration. From then until the

experiment ended they made fair gains. Similarly rat 30A ♂ which replaced rat 30 ♂ gained during the first four weeks and then lost weight until the butter fat was added. The slow growth of all the animals and the death of two of them were due in a large degree to the lack of the fat soluble vitamine. The results are quite satisfying, however, when the unbalanced nature of the ration and the toxicity of the raw seeds are considered.

TABLE 6

Growth of rats on cotton seeds and salt mixture

	WEIGHT				
	Rat 27 ♀	Rat 28 ♂	Rat 29 ♂	Rat 30 ♂	Rat 30A ♂
	grams	*grams*	*grams*	*grams*	*grams*
February 21	49	57	57	78	
April 16	71	71	75	85 (Died)	
May 20	75	85	92 (Died)		78
June 26	62	71	(Butter fat added)		78
August 1	92	113			100
Gain	+43	+56	+35	+7	+22

These results lead us to believe that the untreated cottonseed meal is quite toxic when fed in too large quantities or when fed to young animals. Yet by proper treatment, it may be prepared so that it becomes an excellent source of protein for supplementing protein deficient rations.

CONCLUSIONS

1. Cottonseed meal can be made a safe feed for swine, and probably other livestock as well, by autoclaving or steaming the meal until it is thoroughly cooked.

2. Such treatment renders the meal more palatable and in so doing raises its value as a feed.

3. A deficiency in the ration cannot account for the difference in the gains made by pigs receiving the untreated cottonseed meal and those receiving the cooked meal.

4. Supplements such as $CaCO_3$, yeast, and butterfat when

used to produce a balanced diet do not overcome the toxic effect of either the cotton seeds or the meal when fed to albino rats.

5. Animals may make small gains extending over a considerable length of time on rations containing a limited amount of cottonseed meal—above that limit they lose weight and usually die if the feeding is continued.

6. Cooking the meal increases the amount which may be safely fed over any period of time.

In conclusion the author expresses his thanks to P. L. Menaul for many helpful suggestions during the progress of this work.

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ENERGY REQUIREMENTS OF DAIRY COWS

A REPLY TO ARTICLES BY E. B. MEIGS AND H. T. CONVERSE

E. B. FORBES

From the Institute of Animal Nutrition of the Pennsylvania State College

In this JOURNAL for May, 1925, appeared an article by E. B. Meigs and H. T. Converse, on the Quantities of Nutritive Energy Necessary to Maintain Dairy Cows in Nutritive Equilibrium, in which the authors presented a critical comparison of the feeding standards for maintenance of dairy cows, as proposed by Henry and Morrison, by Eckles, and by Armsby; and also a comparison of digestible nutrients and net energy, as measures of the nutritive value of feeds; and in the November, 1925, number of this JOURNAL appeared a second article, by E. B. Meigs, on the energy requirements of dairy cows, under the title, Is Net Energy or Metabolizable Energy the More Useful Index for Practical Purposes.

Referring first to the second article, this consists of a criticism of the respiration experiments of Kellner and Armsby—the two preëminent scholars in the field of energy metabolism of farm animals. In discussing Armsby's work, however, Dr. Meigs bases his criticisms and conclusions mainly on items selected from his earlier, pioneer studies which are no longer fairly representative of the work of this Institute. All of this older work has been recalculated and revised in the light of recent advance in methods and understanding.

Since Armsby's death this Institute continues its program of measurement of the nutritive values of feeds, in terms of energy, adhering to the fundamental conceptions advanced by Armsby, and employing the most elaborate methods of research in use anywhere in the world. It stands committed to no particular figures for nutritive value, or nutritive requirements. The published figures for nutritive values and requirements may have to be revised again and again before the best possible results are

finally obtained; but we are committed to the principle of determining the nutritive value of feeds, in terms of energy, by the subtraction from their gross energy (fuel value) the total of the loses and expenses of feed utilization (likewise, of course, in terms of energy), the results constituting their net energy values.

During recent years—even during recent months—we have made important progress in methods of work, and in spite of an apparently satisfactory grasp of our subject it is not unlikely that we have further important progress in methods still to make. We do not claim, therefore, to have obtained final results, but we do claim that we are investigating the energy value of animal feeds in the most scientific method thus far devised, and we invite the interest and support of those whom we are seeking to serve, with perfect confidence in the successful outcome of these studies of net energy values and requirements.

As for the practical significance of the results, with the obviously necessary allowances for the differences between the standard conditions of scientific study and the varying conditions of scientifically uncontrolled practice, such as are necessary in the application of all exact knowledge, net energy values certainly do apply in practical animal production.

Referring now to the first article—in the experiment by Dr. Meigs upon which this article is based there appear not to have been even so much as feed analyses, to say nothing of digestion trials—in other words, all of his data for nutritive values and requirements appear to have been computed, or assumed. There are, therefore, in reality, no experimental results to discuss except weights of cows and weights of feeds, which, of course, unsupported by other data, are not competent in relation to fundamental nutritive considerations.

We must insist, therefore, that the conclusions, in terms of digestible nutrients, and net energy, are not in any way warranted by the data presented.

On page 178 of the JOURNAL for May, 1925, Meigs and Converse say, "Armsby bases his standard on an experiment of Kellner in which the complete energy balance was determined for a

milking cow (1, p. 493–500, 511–516)." (These page numbers refer to Armsby's book The Nutrition of Farm Animals.[1])

In a footnote on the same page Meigs and Converse say, "Although Armsby considers the results obtained by Kellner with three cows, he bases his standard on the performance of the most economical of the three, rather than on the average."

This gives the impression, clearly, that Armsby considered no experiments other than Kellner's, and that he based his standard upon absurdly slight and improperly selected evidence.

Armsby explains with great particularity, in the pages cited by Meigs and Converse, the evidence and considerations upon which his standard for milk production was based.

This standard was derived (1) by allowing for maintenance the same amount of energy as for other cattle, and then (2) by computing from Haecker's analyses of milk (par. 604) the equivalents of net energy for fattening, plus 5 per cent, for the production of milk of the different grades as to fat content, in the light of the following assumptions (*a*) that digestible carbohydrate and protein in the feed are converted into the corresponding compounds of milk without loss, and (*b*) that the expenditure of energy in the production of milk fat from carbohydrates is the same as that observed by Kellner for the production of body fat (par. 593).

The evidence which Armsby considered and which he discussed as contributing to the establishment of this standard is, in the main, as indicated below:

1. The standard for maintenance was adopted in consideration of results of Armsby and Fries, Kellner, Haecker, Evvard, and Eckles (par. 381).

2. Conclusions as to utilization of feed protein in the synthesis of milk protein were based on results of experiments by Jordan, Hayward, Kellner, Hart and Humphrey, Haecker, and Royal Vet. and Agr. High School, Laboratory for Agricultural Research (Copenhagen) (par. 586).

3. In relation to the requirement of protein in the ration

[1] Armsby, H. P. 1917 The Nutrition of Farm Animals, 743 p., illus., New York.

Armsby also considered the effects of the plane of protein intake on the production of milk, as indicated by the results and conclusions of Jordan, and Morgen (par. 599), Wolff and Lehmann, Woll, and Phelps (par. 601), and Haecker (par. 602).

4. Conclusions relating to the utilization of energy for milk production were based on three complete energy balances by Kellner (par. 589), four partial balances by Jordan (par. 590), and also a consideration of Haecker's nine years' results of feed, computed digestible nutrients, live weight, and milk production, with the Minnesota Station herd of cows (par. 590).

5. The addition of 5 per cent to the equivalent energy for fattening, for each grade of milk, rests upon Eckles's finding (par. 722) that with well-fed cows the digestion coefficients were, on the average, 5 per cent lower than those which have been used in computing net energy values.

In spite of the very careful study upon which this standard was based Armsby regarded it as only tentative, as is shown by his own comment, as follows:

> By this device of reducing the total energy content of the milk to the equivalent amount of net energy for fattening, it appears possible to utilize the net energy values of feeds obtained by Kellner and others in maintenance or fattening experiments as a basis for computing rations for milk. Such a method is, of course, provisional, and the basis for it at present is somewhat slender, but it seems the best one now available.

Kellner's energy balances with three cows, were used by Armsby, along with much other evidence, as indicating the higher utilization of energy for milk production than for fattening, and as confirmatory of the hypothesis in accord with which he explains the higher net energy values of feeds for milk production than for fattening.

These energy balances of Kellner, therefore, were contributory only in an indirect and general manner, and do not enter at all into the computation of Armsby's standard.

The energy requirements for milk production, according to Armsby's standard, are expressed not in terms of percentages of

utilization of metabolizable energy but in terms of net energy equivalents for fattening; and this standard calls for much more net energy (for fattening) per 100 calories of milk energy than the amounts indicated by the results of Kellner's experiment. This is clearly shown by a comparison of the data in tables 1 and 2.

TABLE 1

Energy equivalents for fattening per 100 calories of milk energy as indicated by the results of Kellner's experiments

	NET ENERGY FOR FATTENING PER 100 CALORIES OF METABOLIZABLE ENERGY	MILK ENERGY PER 100 CALORIES OF METABOLIZABLE ENERGY	EQUIVALENTS OF NET ENERGY FOR FATTENING PER 100 CALORIES OF MILK ENERGY
	calories	*calories*	*calories*
Cow A	48.02	68.41	70.2
Cow C	46.35	72.80	63.7
Cow E	43.81	66.91	65.5

TABLE 2

Energy equivalents for fattening for different grades of milk according to Armsby's standard

GRADE OF MILK	ENERGY CONTENT PER POUND	EQUIVALENT NET ENERGY FOR FATTENING PER POUND	EQUIVALENTS OF NET ENERGY FOR FATTENING PER 100 CALORIES OF MILK ENERGY
per cent fat	*calories*	*calories*	*calories*
3.0	278	214	77.0
4.0	336	265	78.9
5.0	390	315	80.8
6.0	440	361	82.1
7.0	492	408	82.9

All figures except the last column in both tables are quoted from pages (494, 495, 511 and 513) of Armsby's book which are included in the citation by Dr. Meigs.

The results obtained with Kellner's most efficient cow (cow C) show a net energy equivalent for fattening as low as 63.7 calories per 100 calories of milk energy, while according to Armsby's standard the net energy equivalents for fattening, per 100 calories of milk energy, range from 77.0 calories to 82.9 calories for the different grades of milk, as indicated in table 2.

Thus, in the light of Armsby's own explanation of how he arrived at his conclusions the following statements of Meigs and Converse are clearly erroneous. "Armsby bases his standard on an experiment of Kellner in which the complete energy balance was determined for a milk cow. . . . . " "Although Armsby considers the results obtained by Kellner with three cows, he bases his standard on the performance of the most economical of the three, rather than on the average."

VITAMIN STUDIES

XIII. VITAMIN B IN EVAPORATED MILKS MADE BY VACUUM AND AERATION PROCESSES*

R. ADAMS DUTCHER AND EMMA FRANCIS

Department of Agricultural and Biological Chemistry

AND

W. B. COMBS

Department of Dairy Husbandry, Pennsylvania State College

The investigational work described in this paper is the first of a series of coöperative studies being made at the Agricultural Experiment Station of the Pennsylvania State College on evaporated milks. This work is a continuation of a larger project concerning the various factors influencing the vitamin content of milk and dairy products. Former work has dealt with dietary factors (1) (2), heat and pasteurization (3), oxidation (3) (4), and commercial drying by the spray process (5).

In the past practically all of the data available, relative to the vitamin content of evaporated milks, have been obtained on samples the past history of which has not been known. Furthermore, few attempts have been made to compare the evaporated milks with the raw milks from which they were made. As a result few authoritative data have been obtained concerning the influence of commercial evaporation processes on the stability of the various vitamins.

The present studies were undertaken with the view of preparing evaporated milks under conditions approximating, as closely as possible, the conditions used in commercial practice and comparing these products with the original milk from which they were made, to eliminate the effect of any fluctuation in vitamin content that may take place in raw milk obtained from various sources and at different seasons of the year.

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THE EVAPORATED MILKS

The raw milk, from which the evaporated milks were made, was obtained, daily, from the college herd and was fed to the experimental animals within a few hours after the morning milking, although a portion of the raw milk represented the milking of the previous evening.

Two types of evaporated milk were prepared, the first being manufactured by the vacuum process in a Rogers 18-inch copper vacuum pan and the other by the aeration method, passing a current of air through the hot milk. The object in using the methods just described was to determine, if possible, the susceptibility of vitamins to oxidation during the evaporation process. We also desired to study the effect of subsequent sterilization, for sterilization plays an important rôle in the manufacture of commercial evaporated milks. At this point it is well to emphasize the fact that the writers do not claim to have duplicated commercial methods of manufacture but the attempt has been made to approximate commercial conditions as closely as possible.

EVAPORATED MILK MADE BY THE VACUUM PROCESS

While the raw milk was fed daily to the experimental animals, it was impossible to manufacture daily "batches" of evaporated milks. As a result the evaporated milks used in this study were manufactured every three weeks.

Prior to the evaporation process, milks from the evening and morning milking were mixed thoroughly and samples were taken for analysis. These samples were analyzed for titratable acidity, total solids and fat. The specific gravity was recorded also.

In the case of milks made by the vacuum process, the raw milk was pre-heated in the hot-well until the temperature reached 200° to 210°F., after which it was drawn into the vacuum pan. The temperature in the pan ranged from 125° to 135°F., averaging about 130°F. The average vacuum obtained was about 23 inches. Unfortunately the available supply of raw milk was occasionally limited, with the result that the charges in the pan

were not constant in volume; as a consequence, the time of evaporation varied from forty-five minutes to one hour and fifteen minutes.

When the milk in the pan had reached a concentration of 7° to 9° Beaumé at 120°F., it was homogenized at 1800 to 2000 pounds pressure. Specific gravity, solids and fat were determined on the homogenized evaporated product after which one half of the evaporated milk was placed in sterilized bottles, of 250 cc. capacity, and these bottles were placed on ice until needed. Each bottle usually contained sufficient milk for one day's feeding.

The other half of the evaporated milk was also placed in similar bottles, plugged with cotton and sterilized at 240°F. for ten minutes and at 225°F. for five minutes, using a laboratory autoclave. These bottles were also placed on ice until needed for feeding purposes.

By the use of the analytical data, described above, it was possible to dilute the evaporated milk samples with distilled water until the diluted milks contained solids and fat equal to that of the raw milk from which they were made. All evaporated milks were fed daily in the diluted state in direct comparison with fresh raw herd milk.

EVAPORATED MILK MADE BY THE AERATION METHOD

During the early part of the investigational work the aerated evaporated milks were made in a Ruff evaporator of the commercial type, consisting of a heated revolving drum which was partly immersed in the milk. Air was driven through the heated milk by means of blower pipes. The experimental milk, used in this study, was produced by the college dairy herd that furnished the local market with certified and grade A milk. As a result there were periods when the amount of milk available for evaporation purposes was so limited that the quantity of milk was insufficient to immerse the heated drum. For this reason the use of the Ruff evaporator was abandoned and air pipes were connected with a glass lined pasteurization tank of small capacity and the subsequent aerated evaporated milks were made by this method, heating the milk by means of steam.

The hot-well temperatures were maintained at 145°F. and the condensing temperatures ranged from 130° to 155°F. On account of the varying quantities of fresh milk, the time of condensing varied from forty-five minutes to one hour and fifteen minutes.

From this point the evaporated milks made by the aeration methods were treated the same as those made by the vacuum process; i.e., homogenized and a portion sterilized by the fractional sterilization method.

During the entire period the college herd was confined to the barn and yard, without pasture, and received a ration relatively constant in composition with the exception that some green hay was fed during two months in the fall. The ingredients of the ration, expressed in parts per 100, were as follows:

	Per cent
Wheat bran	12
Barley	12
Oats	8
Hominy	24
Cotton seed meal	12
Linseed meal	16
Peanut meal	16

Some minor modifications were made in the proportions of the above ingredients during the latter part of 1924. During the entire period mixed hay and silage were fed, as roughage, with exception of the two months mentioned above, when green hay was fed.

It will be seen, therefore, that the vitamin B intake of the herd was about as constant as can be expected under ordinary feeding conditions.

It will be noted that five types of experimental milks were fed, which we have abbreviated for convenience (on the figures) as (*a*) R, i.e., raw milk; (*b*) V.E., i.e., vacuum evaporated milk (not sterilized); (*c*) V.E.S., i.e., vacuum evaporated milk which had been sterilized; (*d*) A.E., i.e., aerated evaporated milk (not sterilized); and (*e*) A.E.S., i.e., aerated evaporated milk which had been sterilized.

THE FEEDING EXPERIMENTS

A total of 289 rats have been used in this work, although the charts do not represent that number, owing to the fact that it was necessary to repeat some of the early work on account of the observations of Steenbock, Sell and Nelson (6) and Dutcher and Francis (7) that vitamin B studies are inaccurate unless provisions are made to eliminate the possibility of the rats having access to their own excretory material. This error was eliminated in part at least, by changing our feeding technique and adopting the use of false cage bottoms consisting of wire screen (3 or 4 meshes to the inch). Since the publication of the above mentioned findings Salmon (8) and Smith, Cowgill and Croll (9) have published experimental data substantiating the work of Steenbock, Dutcher and co-workers and showing that this change in feeding technique is necessary if the best results are to be obtained. In our publication (7) we stated that we were of the opinion that "the rat is limited in its ability to store vitamin B." As a result it was decided that little could be gained by using the "curative" method of feeding and, consequently, all rats described in this paper were fed the various experimental milks from the beginning of the experimental period, rather than adding the milk to the diet after vitamin deficiency had become evident, which is the practice in the "curative" method.

RAT RATION AND FEEDING TECHNIQUE

The ration consisted, in parts per 100, of casein 18, salts 3, agar 2 and dextrin 77. Four drops of a potent cod liver oil were fed daily, separate from the ration, to insure an adequate intake of vitamins A and D. Iodine was furnished in the drinking water. The casein was prepared by precipitating skim milk according to the Zoller method (10). The washings, with acidified water (pH 4.8), never numbered less than 12 and between washings the casein was pressed as free of water as possible, in a cheese press.

The water washings were followed by 5 or 6 alcohol washings, allowing each "batch" to remain in contact with alcohol for at least three hours. This treatment served to extract any traces

of vitamin B remaining in the casein and, incidentally, removed sufficient water to prevent spoilage of the casein on standing. After treatment with alcohol the casein was spread in thin layers on glass shelves in an air drier and air (at room temperature) was driven over the casein to remove the alcohol.

The casein was then treated with ethyl ether until 50 cc. of the ether extract left no fatty residue, upon evaporation. The casein was then air dried, heated in an air oven at 120°C. for twelve hours and ground to a powder. This product has been used in all of our feeding work and has been found to be free from vitamins A, B and C.

The dextrin was prepared by autoclaving corn starch in the moist state, in the presence of 0.4 per cent citric acid. After drying in a steam closet, until the product was thoroughly brittle, it was ground to a fine powder.

The salt mixture was McCollum's salt mixture No. 185 and the agar was a high grade product of the type used in bacteriological work.

As is the custom in this laboratory, all experimental animals were confined in individual cages and food consumption records were obtained on every individual. These food intake records were used as a guide in the interpretation of data but are not of sufficient importance to be included in the charts.

Each experimental group contained not less than 8 individuals and some groups (particularly the groups receiving 12 cc. of milk) contained as many as 16 rats. Males and females were distributed equally in all groups to eliminate the influence of sex on the growth curves obtained in averaging the individual growth curves in each group. On account of the large number of animals, it was thought best to conserve space by presenting "group averages" rather than growth records of each individual. It was felt, also, that the data are more easily interpreted by consulting averages for each group.

All feeding experiments were conducted for one hundred forty days. In order that seasonal variations, if any, might be eliminated all animals were not placed on experiment at one time but groups were added from time to time during the experiment.

When this was done, however, an equal number of rats were placed in each experimental group.

Osborne and Mendel (11) have shown that a minimum of 16 cc. of milk, produced under conditions existing at New Haven, were needed to furnish sufficient vitamin B for the normal growth of rats. The work of Kennedy and Dutcher (2), working with milk produced under feeding conditions existing in St. Paul, found that they could obtain satisfactory growth of rats on as little as 10 cc.

With these results in mind, it was planned to feed the milk, described in this paper, at levels below and above 10 cc. Consequently, the milk was fed at 6, 8, 10 and 12 cc. levels with the hope that we would find a "threshold level" at which point vitamin destruction (if any) by heat or oxidation would be most marked.

DISCUSSION

All of the experimental data have been averaged and are summarized in figures 1 and 2. None of the animals receiving 6 cc. of milk grew normally, although growth was quite satisfactory.

No outstanding differences seem to exist between the groups receiving 6 cc. of raw milk and those receiving equivalent amounts of vacuum evaporated (V.E.) milk. Furthermore, the sterilization of this evaporated milk did not seem to affect its nutritive properties. The rats receiving 6 cc. of the aerated (A.E.) evaporated milk did not grow so well but the differences are not marked. Sterilization of this evaporated milk did not bring about differences sufficiently large to merit emphasis.

What has been said for the rats receiving 6 cc. of milk can be said, also, for those fed at the 8 cc. level. In fact, no effect of the additional 2 cc. of milk can be noted on any of the curves.

When the volume of milk is increased to 10 cc. it is possible to note a distinct improvement in all groups. It is difficult, however, to note any effect of milk treatment in any of the groups receiving more than 6 cc. of milk.

When the volume of milk, fed per rat, was increased to 12 cc. fairly normal growth was obtained, showing that the Pennsyl-

vania herd milk was slightly superior, in vitamin B content, to that described by Osborne and Mendel (11) but it seems slightly inferior to the Minnesota milk described by Kennedy and Dutcher (2).

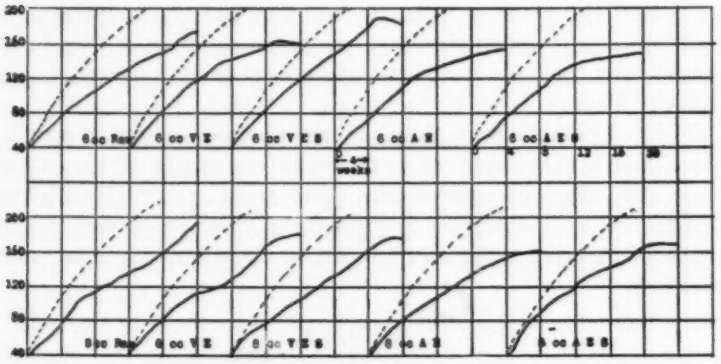

FIG. 1

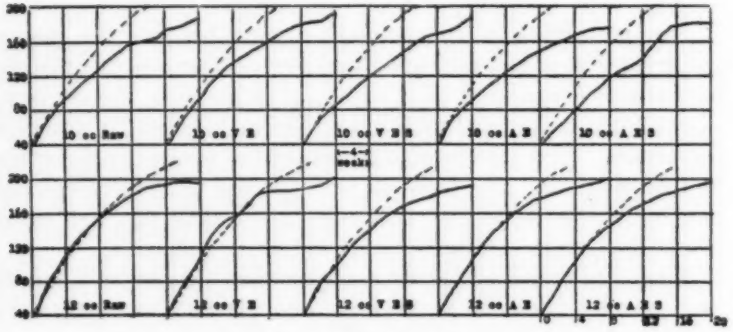

FIG. 2

SUMMARY AND CONCLUSIONS

Evaporated milks have been made by vacuum and aeration methods, approximating commercial manufacturing conditions as closely as possible. These milks were sterilized and compared, by animal experiments, month by month, with unsterilized evaporated milk as well as with the raw milk from which they

were made. This was done to study the effect of heat and oxidation on the stability of vitamin B. The evaporated milks were diluted with distilled water until they possessed the same composition as the raw milk and all experimental milks were fed at 6, 8, 10 and 12 cc. levels. No evidence of vitamin B destruction could be noted except in the groups fed at the 6 cc. level. In these groups there was some evidence that aeration or oxidation and heat may have brought about slight destruction of vitamin B. The destructive effect, however, was so slight that it is doubtful if it can be considered to be of nutritional importance.

We are forced to conclude, therefore, that vitamin B is not readily destroyed by the evaporation methods described and only under unusual conditions would we expect the vitamin B deficiency of commercial evaporated milks to be due to methods of manufacture.

Experimental work is now in progress to attempt to determine the stability of vitamin A during the processes of evaporation and sterilization by the methods described.

The writers wish to acknowledge the assistance of Miss Mattie Creighton, who assisted materially in the experimental phases of the work, and the hearty coöperation of the herdsman, Mr. P. D. Jones.

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THE EFFECT ON MILK PRODUCTION OF FEEDING MORE THAN THE HAECKER, ECKLES, AND SAVAGE REQUIREMENTS*

H. T. CONVERSE[1]

Research Laboratories of the Bureau of Dairying, United States Department of Agriculture

Haecker (1) and Eckles (2), in their studies of the feed requirements for milk production, determined the amount of nutritive energy necessary to maintain milking cows at uniform body weight. Savage (3) has published a standard in terms of protein and total digestible nutrients, which is in substantial agreement with the results of Haecker and Eckles as well as with later results obtained at the United States Bureau of Dairying experiment farms, Beltsville, Maryland (4) (5). This last named standard will be used in describing the results to be reported in the following article.

The Savage standard gives the quantities of digestible nutrients necessary to maintain milking cows at uniform body weight. The question remains, however, whether cows will not produce more milk if fed more than this standard demands.

LITERATURE

Several stations have carried out work in which more feed was given in one period than in another. Two types of experiments furnish such data: one studying the effect of varying amounts of grain, and the other studying the effect of grain in addition to roughage.

The New Mexico Station (6) reports two trials of adding grain to a ration of alfalfa hay, and found an increase of 12.2 per cent in production. A study of light versus medium grain feeding

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[1] The author wishes to acknowledge the assistance of Dr. E. B. Meigs of the Bureau of Dairying in the interpretation of results and in the preparation for publication.

was made at the Utah Station (7), with a resulting increase of 8 per cent in production with the heavier grain feeding. In a study on "Heavy versus light grain feeding for dairy cows," the California Station (8) found an increase in production of 5 per cent on the more liberal ration. In all these trials the less favorable rations were supernormal, yielding digestible nutrients from 12 to 45 per cent in excess of the Savage standard requirements. Thus in each case there was an increase in production when an already liberal ration was made more liberal.

Other stations have made somewhat similar reports, but the experiments examined were so conducted as to have little bearing on the question of the effect of feeding at standard as against above standard. All these experiments were purely economy trials, and in but few cases apparently was an attempt made to regulate the quantity of nutrients to the need of the cows.

Eckles and Palmer in well regulated experiments studied the effect of the plane of nutrition on milk yield. In their first paper (9) they conclude that "only in certain cases, which are brought out in the data to be presented later, did an increase in the plane of nutrition above normal raise the flow of milk, and the influence in these cases was very limited." Their conclusion was that in the early months of lactation milk is controlled by a "chemical stimulus" or "hormone" present in the blood and that in this period the plane of nutrition has little if any effect. In the latter part of the lactation period, they conclude that the "chemical stimulus" largely disappears, that the "nervous stimulus" then controls milk secretion, and that here over-feeding does help slightly in holding milk production to a higher level.

These conclusions were based on five experiments in which cows were fed from 15 to 100 days at a supernormal plane of nutrition. A cow overfed for 65 days increased from 13.3 to 15.1 pounds per day. A cow overfed for 30 days increased from 22.5 to 22.9 pounds per day. A cow overfed for 100 days increased from 10 to 11.6 pounds per day. A cow overfed for 15 days increased from 9.4 to 12.9 pounds per day. A cow overfed for 30 days decreased from 20.3 to 18.7 pounds per day.

In a second paper by the same authors (10) on the effect of

underfeeding on milk production, by a similar train of reasoning it is maintained that in the early months of lactation the plane of nutrition—even underfeeding—affects but little, if any, the level of milk production. In the latter stages of lactation the experiments do show clearly a decided decrease in production as a result of underfeeding, and the authors urge the importance of supplying sufficient feed after the "chemical stimulus" imparted at the time of parturition has been lost.

These findings were based on 16 experiments in which cows were underfed for varying periods, usually from 15 to 30 days. These experiments fell into three distinct groups; those starting at calving, those starting about 30 days from calving, and those starting at a considerably later time after calving. In the first group, in which all the cows were overfed before calving and then underfed, three increased slightly in production while three decreased. In the second group two cows, previously underfed, were more severely underfed and decreased in production. The other two cows, also previously underfed, were continued below normal but not so severely underfed as previously and increased in production. In other words they were given more feed, though not a sufficient amount, and increased in production as a result. In the third group six cows, all previously well fed, decreased, some very considerably, in production during the underfeeding period.

Further light on the effects of changes in feed on milk production is to be gained from the reaction to feed changes of certain cows in the study of Cary and Meigs (11) on the "Relation between the diet, the composition of the blood and the secretion of milk in dairy cows." The experiments cited were mostly of three periods of varying lengths. One cow starting about 30 days from calving, after a period on nearly an adequate ration, had both the protein and energy reduced for 37 days and declined in milk production from 12.25 to 7.80 kgm. per day. When placed again on the full ration the decline stopped, and the daily production was increased in 17 days from 8.26 to 9.03 kgm. A second cow 60 days from calving, after a period in which she was fed nearly a sufficient amount, had only the

energy of her ration reduced for a period of 27 days and decreased in production from 11.11 to 9.77 kgm. A slight increase in production was noted in the third period when the displaced energy was returned to the ration. A third cow started 90 days after calving had merely the protein of the ration reduced in the middle period. Here too the production was reduced in the intermediate period and later increased when the ration was again made sufficient.

DISCUSSION OF THE ECKLES AND PALMER RESULTS

That element of the results of the investigation of Cary and Meigs above cited which deals with the effect on production of a ration insufficient to maintain body weight is intentionally compared with the work of Eckles and Palmer, as the results of Cary and Meigs are not entirely in harmony with the conclusions of Eckles and Palmer. The latter authors, as above stated, suggest that neither overfeeding nor underfeeding has an appreciable effect on milk production in the early stage of lactation when the "chemical stimulus" or "hormone" is active and that overfeeding even in the latter stages of lactation has but very small effect.

On the other hand the results of the investigation of Cary and Meigs show that cows even in the first months of lactation are quite susceptible to an inadequate energy supply. Their work also shows that an inadequate supply in either quantity or quality of protein is reflected in the milk flow even in the first 90 days of lactation.

Table 1, which is a portion of the table presented by Eckles and Palmer (10), forms a basis for the discussion of the lack of harmony between the two pieces of work.

From this table it will be noted that in the first group, which consists of cows starting the underfeeding period immediately after calving, the three most heavily underfed actually decline in milk production. The three most lightly underfed do increase somewhat during the underfeeding period. In the second group, consisting of cows starting the underfeeding period 20 to 40 days from calving, the two most lightly underfed before

the experiment and most heavily underfed during the experiment declined in production, while the other two increased in

TABLE 1

Shows the effect of a subnormal plane of nutrition on the milk yield during the first months of lactation

EXPERIMENT	COW	DAILY MILK YIELD		STAGE OF LACTATION	UNDER FEEDING	PLANE OF NUTRITION	
		At first	At end			During experiment	Before experiment
		pounds	*pounds*	*days*	*days*	*per cent*	*per cent*
1	20	22.2	19.9	1	30	−60–70	Not given (?)
2	206	28.4	27.1	1	22	−60	Supernormal
3	301	23.6	16.0	3	30	−38	Supernormal
4	300	35.8	38.9	1	20	−35	Supernormal
5	2	12.3	13.7	4	38	−20–25	Supernormal
6	301	25.1	37.2	5	18	−16	Supernormal
7	2	13.5	8.8	42	36	−30–35	−20–25
8	301	35.3	33.0	24	20	−24.5	−16
9	20	19.6	26.1	32	16	−23	−60–70
10	206	26.1	27.1	23	12	−20	−60

TABLE 2

Shows the average daily milk yield of seven cows by ten-day periods from the time of calving

10-DAY PERIOD	cow 27	cow 62	cow 206	cow 304	cow 400	cow 303	cow 211
	(1)	(1)	(2)	(2)	(2)	(2)	(2)
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
First	29.3	11.6	40.3	36.7	20.8	29.5	39.6
Second	31.0	14.0	42.7	38.9	21.3	30.8	38.6
Third	32.5	14.7	43.3	39.8	22.8	30.3	37.1
Fourth	31.4	15.6	40.9	38.8	22.9	29.3	38.0
Fifth		15.0			22.7	29.7	40.4
Sixth					22.4		41.9
Seventh					22.6		42.4

(1) From Missouri Station, Research Bulletin No. 2. (2) From Missouri Station, Research Bulletin No. 7.

production. However, there appears to be a reason for the improved production of these last two cows. The underfeeding of these cows was drastic before the experiment and much lighter

during the experiment. In other words, there was a raising of the plane of nutrition, and the cows responded to the improved conditions.

During the first few weeks of lactation under adequate feeding the milk yield generally increases considerably. This is shown in table 2 prepared from data of Eckles (12), and Eckles and Reed (13).

In every case the production was lower in the first ten days than in some of the subsequent periods.

Recognizing that this is the case, it would seem that any condition that prevented the normal rise in production would indicate the unfavorableness of that condition. In most of the cases reported by Eckles and Palmer there was not only an arresting of the rise in milk yield due to underfeeding but actually a decline that could be attributed to that cause.

As is well known the curve of normal milk production becomes a declining curve a few weeks after calving. If this fact is taken into account in a study of the data submitted by Eckles and Palmer (9) on the effect on milk production of a supernormal plane of nutrition, it would appear that excess feed makes a very appreciable difference in the milk yield. In four of the five experiments reported by them, there was not only an arresting of the normal decline for those cows in advanced lactation but an actual increase in production due to the overfeeding.

THE EXPERIMENTS

After the decision had been reached that the Savage feeding standard for maintenance and milk production furnished very approximately the amount of feed necessary to maintain uniform body weight, the next logical question was the effect on production of feeding in excess of this standard. Two types of experiments were conducted. The first were comparatively short time experiments, with periods of thirty days in which the cows were alternately fed at standard and above standard. The latter were whole lactation-period comparisons.

TABLE 3

Showing details of weight, production, feed received, and plane of nutrition according to the Savage feeding standard in the first experiment

PERIOD	COW	BREED*	MILK	TEST	BODY WEIGHT	GAIN IN BODY WEIGHT	FEED RECEIVED				PERCENTAGES OF SAVAGE REQUIREMENTS RECEIVED†	
							Grain	Hominy	Alfalfa	Silage	D.P.	T.D.N.
			pounds	*per cent*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*per cent*	*per cent*
(1) At standard	228	H.	322.1	3.40	1,410	−2	50.0	60.0	219.5	1,197.0	105.3	103.3
	236	H.	520.6	3.50	1,095	−29	115.8	38.0	209.5	1,111.0	103.1	101.9
	250	H.	664.4	3.70	1,201	18	158.0	54.0	236.0	1,170.0	100.6	100.4
	420	J.	263.7	5.05	689	2	88.0	17.0	147.0	726.0	106.5	96.5
	435	J.	271.4	5.10	689	5	60.0	74.0	138.5	762.0	102.5	105.3
	84	G.G.	433.8	4.30	816	3	108.2	62.0	170.0	846.0	101.0	100.3
Total			2,476.0			−3	580.0	305.0	1,120.5	5,812.0		
Average				4.00	983						102.5	101.0
(2) Above standard	228	H.	246.6	3.80	1,393	−30	39.6	59.2	300.0	1,144.5	130.5	115.1
	236	H.	474.6	3.90	1,101	41	203.4	8.8	230.0	1,105.0	128.8	113.1
	250	H.	660.5	3.70	1,227	34	307.6	27.4	240.0	1,096.0	131.6	115.1
	420	J.	233.2	5.40	713	45	119.4	36.2	150.0	780.0	131.1	115.6
	435	J.	250.5	5.65	708	35	127.6	48.2	150.0	740.0	129.0	114.4
	84	G.G.	404.8	4.66	825	15	195.2	43.4	180.0	840.0	130.0	114.8
Total			2,270.2			140	992.8	223.2	1,250.0	5,705.5		
Average				4.30	995						130.2	114.6

(3) At standard	228	H.	192.0	3.90	1,373	−10	14.0	95.6	192.0	979.5	104.4	101.2
	236	H.	301.9	4.20	1,110	−23	40.8	90.4	205.0	948.3	104.8	104.3
	250	H.	611.0	3.95	1,243	−2	124.4	112.0	243.0	976.0	99.3	99.4
	420	J.	220.2	6.05	774	18	46.8	60.8	146.0	777.7	100.9	101.0
	435	J.	236.7	6.35	733	13	51.6	61.6	150.0	780.0	99.4	100.0
	84	G.G.	292.1	5.25	831	−2	48.3	64.4	184.0	832.0	100.2	100.0
Total			1,853.9			−6	325.9	484.8	1,120.0	5,293.5		
Average				4.75	1,006						101.3	101.0
(1) and (3) at standard, average			2,164.9	4.33	995	−5					102.0	101.0
(2) Above standard			2,270.2	4.30	995	140					130.2	114.6

Average increase in milk production of the above standard period over the standard period, 4.9 per cent.

* Breed: H., Holstein; J., Jersey; G.G., Grade Guernsey.

† D.P., digestible protein; T.D.N., total digestible nutrients.

First experiment

In this experiment six cows were fed for three periods of thirty days each. In the first and third periods they were fed as nearly as possible at Savage requirements, while in the second period they received 30 per cent more protein and 15 per cent more digestible nutrients than is required by the Savage standard. The protein was regulated by the addition of hominy feed to the basal grain ration consisting of hominy feed 2 parts, ground oats 2 parts, wheat bran 2 parts, linseed-oil meal 1 part, and cottonseed meal 1 part. Because the cows were far advanced in lactation no transition period was allowed between the experimental periods.

The feed allowed for maintenance was based on three-day body weights taken at the start of each experimental period. The feed allowed for production was based on the actual milk production and the fat test of a two-day composite taken at the middle of each ten-day subperiod. In other words, the feed allowed for production for each thirty-day period was based on the fat tests of three two-day composites taken at ten-day intervals. The results of the first experiment are shown in table 3.

In this trial cows that had been milking 18, 13, 11, 11, 9, and 6 months respectively and producing as little as would be expected at these stages of lactation increased on the average 4.9 per cent in milk production when fed 15 per cent in excess of Savage requirements for total digestible nutrients. It will also be noted that on the average the cows lost only 5 pounds in body weight per thirty days when fed at Savage requirements. This is a little less than one pound per cow.

Second experiment

In this experiment five cows were fed for three thirty-day periods with ten days between for transition. In the first and third periods it was planned for the cows to receive the Savage requirements, while in the second or intermediate period they were to get 20 per cent protein and 10 per cent total digestible nutrients in excess of Savage requirements. In other respects,

this experiment was conducted in exactly the same way as the first experiment. The results of the second experiment are shown in table 4.

In this trial the cows were about as far advanced in lactation as were those in the first trial, having been fresh 13, 13, 12, 11, and 7 months respectively. The cows were fed close to the planned amounts, both in the at-standard and the above-standard periods. The increase in milk production was but 1.9 per cent.

It will be noted that in these two experiments the percentage gain in production was quite small; 4.9 per cent in the first, and 1.9 per cent in the second. The average for the two experiments gives but 3.3 per cent actual increase in production. A more accurate index of the value of the supernormal ration is seen in the decline in milk yield under the two conditions. In chart 1 the average daily milk yield has been computed for all cows in both experiments by five-day subperiods, and the decline has been plotted for the two periods at standard feeding as well as for the intermediate period when fed above standard. This chart also gives the same information for the two experiments separately, each showing fairly close agreement with the combined chart.

It will be seen that at standard the cows declined nearly 20 per cent in the thirty-day periods, while at above standard there was no decline in production. In fact, in every five-day subperiod when the cows were fed above standard the yield was higher than in the last five-day subperiod when fed at standard.

From a study of the curves given in chart 1, it is clear that simply comparing the total amounts of the milk yield of the period when the cows were fed above standard with those of the preceding and subsequent periods when they were fed at standard does not give a fair quantitative idea of the effect of the surplus feed on the milk yield. On account of the large amount of feed given in the middle period, the cows start the third period at a higher level of milk yield than would have been the case if they had been fed at standard all along; in other words, a considerable part of the milk given in the third period

TABLE 4

Showing details of weight, production, feed received, and plane of nutrition according to the Savage feeding standard in the second experiment

PERIOD	COW	BREED*	MILK	TEST	BODY WEIGHT	GAIN IN BODY WEIGHT	FEED RECEIVED				PERCENTAGE OF SAVAGE REQUIREMENTS RECEIVED†	
							Grain	Hominy	Alfalfa	Silage	D.P.	T.D.N.
			pounds	*per cent*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*per cent*	*per cent*
(1) At standard	50	G.H.	751.8	4.00	1,206	−33	152.0	65.6	300	1,200.0	100.0	100.2
	248	H.	1,003.2	3.70	1,192	−2	241.2	65.6	299	1,200.0	99.7	99.9
	416	J.	247.7	5.40	873	17	41.8	51.2	170	900.0	99.6	99.6
	427	J.	299.2	5.30	709	40	68.8	52.0	146	780.0	97.1	97.7
	443	J.	234.3	4.90	618	26	48.4	36.0	138	714.0	100.5	98.9
Total			2,536.2			48	552.2	270.4	1,053	4,794.0		
Average				4.25	920						99.5	99.4
(2) Above standard	50	G.H.	763.9	4.10	1,203	26	300.0	61.5	257	1,072.5	120.3	109.9
	248	H.	978.0	3.80	1,204	28	366.2	40.6	299	1,118.9	119.6	109.4
	416	J.	227.9	5.40	893	24	87.3	44.1	169	891.5	119.7	109.1
	427	J.	282.9	5.95	741	24	131.4	53.6	150	772.5	120.7	110.2
	443	J.	184.6	4.80	657	51	56.4	38.6	150	720.0	120.7	110.2
Total			2,437.3			153	941.3	238.4	1,025	4,575.4		
Average				4.37	940						120.2	109.8

(3) At standard	50	G.H.	782.9	4.30	1,204	−22	208.2	119.2	239	1,033.0	100.3	100.5
	248	H.	956.1	3.95	1,202	−33	207.0	118.4	298	1,038.0	96.8	98.1
	416	J.	194.4	6.03	901	−9	23.8	56.6	170	900.0	100.3	100.4
	427	J.	184.4	7.45	768	29	45.6	55.6	150	779.0	101.6	99.3
	443	J.	130.8	5.85	691	17	9.4	44.0	147	720.0	104.3	101.2
Total			2,248.6			−18	494.0	393.8	1,004	4,470.0		
Average				4.65	953						99.8	99.7
(1) and (3) at standard; average			2,392.4	4.44	937	15					99.6	99.6
(2) Above standard			2,437.3	4.37	940	153					120.2	109.8

Average increase in milk production of the above standard period over the standard period, 1.9 per cent.

* Breed: H., Holstein; J., Jersey; G.H., Grade Holstein.

† D.P., digestible protein; T.D.N., total digestible nutrients.

ought really to be credited to the surplus feed given in the second period. A better idea of the true relations can be given

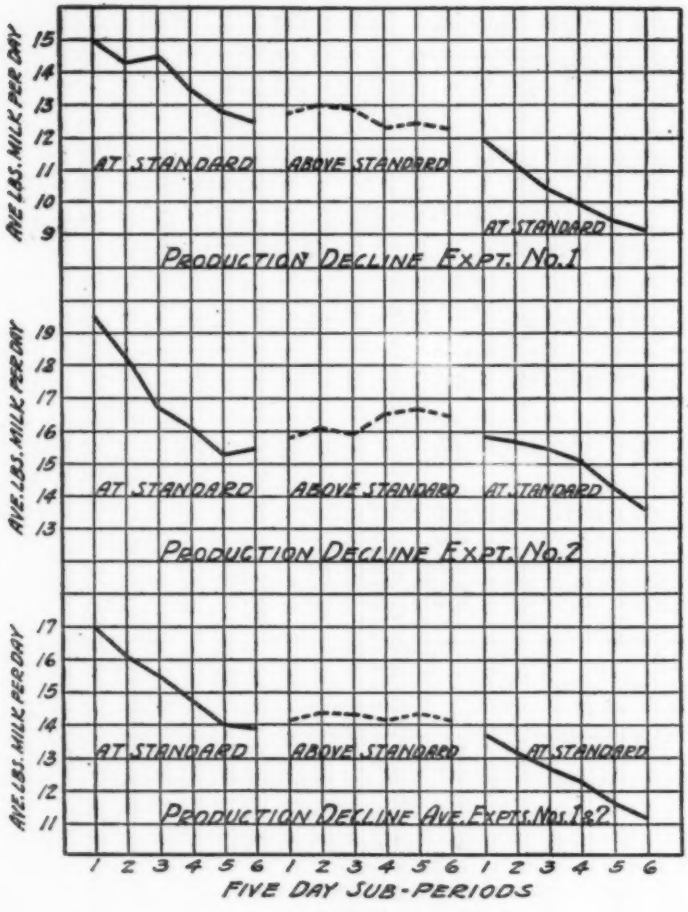

CHART 1

by comparing the actual milk yield of the second period with what would have been the yield if the decline had been as rapid

as it was in the first and third periods. On this basis of comparison, the surplus feed caused an increase of about 16 per cent over what would have been the yield if the cows had been fed at standard in the second period.

TABLE 5

Summary of third experiment, showing comparative results of two lactations on six cows when fed above and at standard

COW	AGE		LENGTH OF LACTATION	LENGTH OF PREGNANCY	PERCENTAGE RECEIVED OF SAVAGE REQUIREMENTS (T.D.N.)	MILK PRODUCTION	DECLINE IN MILK, FIRST TO LAST MONTH	PERCENTAGE DECLINE IN MILK PER MONTH	DAYS DRY BEFORE LACTATION
	years	*months*	*days*	*days*	*per cent*	*pounds*	*per cent*	*per cent*	
236	2	3	330	0	116.7	12,513.4	31.3	3.9	First lactation
236	4	8	303	201	101.3	11,654.3	65.2	8.2	80
255	2	9	365	215	113.3	11,202.2	34.9	3.5	First lactation
255	3	11	365	192	101.1	10,670.0	73.3	7.3	52
444	2	5	365	214	105.9	9,553.6	33.7	3.4	First lactation
444	3	7	365	218	99.8	9,157.1	64.2	6.4	62
442	3	3	331	154	122.4	6,567.3	77.2	8.6	114
442	4	7	331	209	100.3	8,088.4	82.0	9.1	85
443	3	0	336	148	126.8	6,680.9	68.4	7.6	86
443	4	3	336	209	100.6	7,828.2	52.1	5.8	46
465	6	10	365	176	121.9	8,411.7	60.4	6.0	Over 90
465	8	1	365	2	102.6	6,388.7	64.0	6.4	82
Average above standard.....	3	5	344	151	117.8	9,154.9	49.0		
Average at standard.....	4	10	344	172	101.0	8,964.5	68.0		

T.D.N., total digestible nutrients.

Third experiment

Many cows at this station are placed on 365-day semi-official tests. They are kept in boxstalls, milked three times a day, and fed considerably above the requirements of the Savage

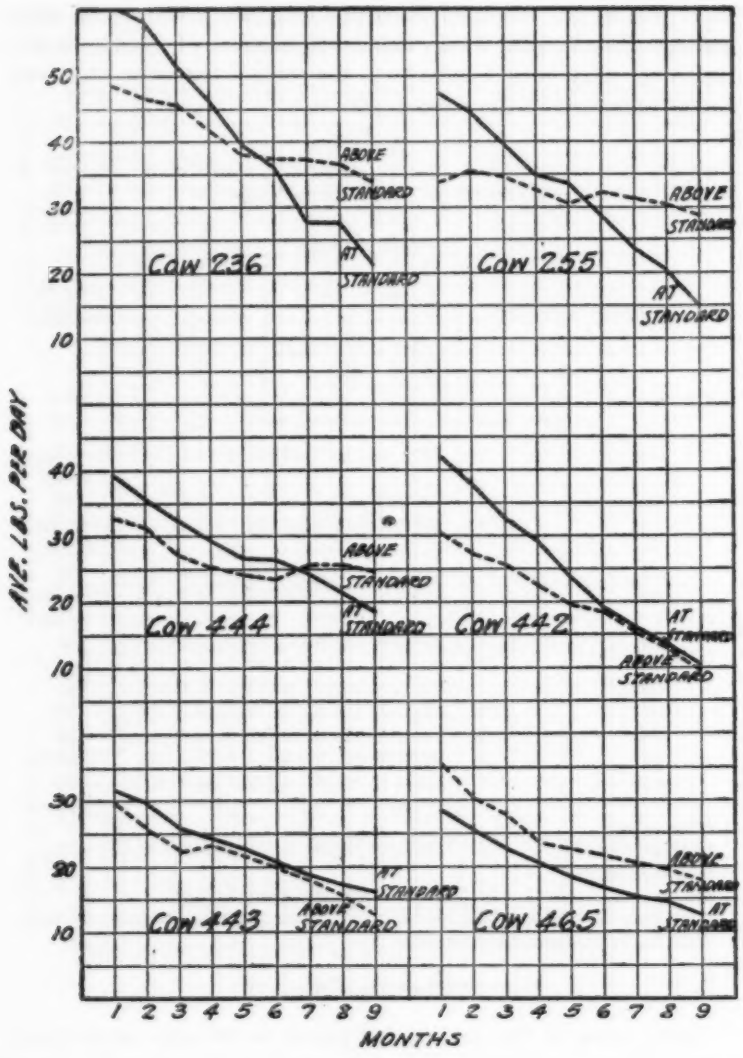
AVE. LBS. PER DAY
50
40
30
20
10
COW 236
ABOVE STANDARD
AT STANDARD
COW 255
ABOVE STANDARD
AT STANDARD
COW 444
ABOVE STANDARD
AT STANDARD
COW 442
AT STANDARD
ABOVE STANDARD
COW 443
AT STANDARD
ABOVE STANDARD
COW 465
ABOVE STANDARD
AT STANDARD
1 2 3 4 5 6 7 8 9
1 2 3 4 5 6 7 8 9
MONTHS

CHART 2

standard. Six of these cows were placed in the succeeding lactation under the same conditions and their feed regulated as nearly as possible to meet exactly the Savage standard.

In three cases, due to early conception, the second or at standard lactation had to be stopped at less than a year. In these cases comparisons are made with an equal portion only of the first or above standard period. Thus the two lactations for each cow are of the same length. Table 5 gives the results of this

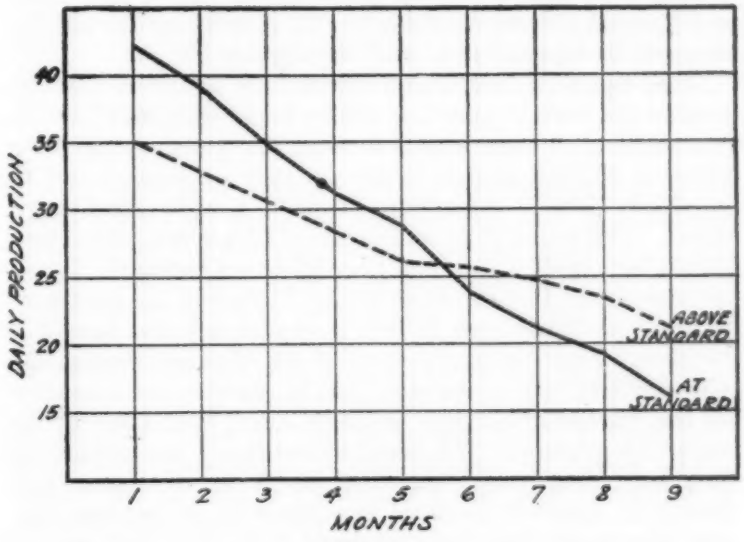

CHART 3

experiment, showing the comparative milk production, feed received, and decline in production for each cow under the two levels of feeding. The table also gives the time when the cows became pregnant, their ages at the beginning of the lactation periods and the length of time each cow was dry between lactation periods.

Chart 2 shows the decline in average daily milk yield for each cow for the first nine full months of the lactations compared. Chart 3 shows the average decline for the whole group.

From Table 5 it will be seen that the cows gave 2 per cent less milk when fed at standard than when fed above standard. They were pregnant for 21 days longer on the average in the lactation at standard, but were 1 year and 5 months older. They were all at a period of life when, other things being equal, they would have been expected to give more milk at the more advanced age.

Graves and Fohrman (14) give figures from which the increase in milk yield with advancing age in heifers can be calculated; and Ragsdale, Turner and Brody (15) give figures for the decrease to be expected as a result of pregnancy.

If the figures of Graves and Fohrman are applied to the milk yield of the cows in table 3 it will be found that, based on the production at 3 years and 5 months, the group should have averaged 10,288.6 pounds in the lactation at 4 years and 10 months in place of the average of 8964.5 pounds actually produced. This would show an increase of 15 per cent which they should have made if they had been fed above standard. It will be remembered that in the at-standard lactation the cows were pregnant on the average 21 days longer than in the preceding lactation. From the figures given by the Missouri investigators above referred to it would seem that an allowance of 4 per cent for this fraction of a month would be a very liberal one for the extra time pregnant. This would be less than 1 per cent for the lactation. Thus stated quantitatively it would appear that feeding at standard produced approximately 14 per cent less milk than would have been produced if the cows had been as highly overfed in the second lactation as in the first.

The comparative decline in production in the two lactation periods as shown in charts 2 and 3 is another way of indicating that feeding cows just at standard does not produce the maximum milk yield. Only one of the six cows decreased more rapidly when fed above standard than when fed at standard, and that difference was but slight as compared with the difference in the opposite direction of several of the other cows. Four of the cows decreased decidedly more rapidly when fed at standard, as was the case in the short time experiments. A comparison

of the graphs of cow 236 and cow 255 is of especial interest. Cow 236 was not pregnant during her first lactation, yet was pregnant 201 days in the second or at standard lactation. The more rapid decline in production in that lactation might be attributed to pregnancy but for the fact that cow 255 showed declines very similar to those of cow 236, with length of pregnancy just slightly greater in the at-standard lactation. If the cows in the third experiment had declined in milk production no more rapidly in the second lactation than they did in the first, they would have produced nearly 16 per cent more milk than they did when fed only at standard. Thus figured in two ways, the value of the overfeeding in this experiment was about 14 per cent and 16 per cent respectively.

SUMMARY

The results taken together indicate that feeding cows according to the Savage or Haecker standard, that is, so that they will just maintain uniform body weight, does not keep them at their maximum milk yield. The three experiments cited are in close agreement in showing that an above standard ration very materially increases production. The two short time experiments with cows well along in lactation give a 16 per cent increase in milk yield as the result of feeding 12 per cent more than the requirements; while the third experiment in which whole lactation period comparisons are used, showed from 14 per cent to 16 per cent increase in production as the result of feeding 17 per cent more than the requirements.

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DAIRY NOTES

THE FUMIGATION OF CHEESE BY HYDROCYANIC ACID GAS

In the JOURNAL OF DAIRY SCIENCE (viii: 287–289, May, 1925), there appeared an article by Perez Simmons entitled "Hydrocyanic acid retained by fumigated cheese." This paper gives the authors' experimental data on the fumigation of cheese, which are the basis for his criticism of the recommendation for fumigation practice as given in California Agricultural Experiment Station Bulletin 343 (May, 1922) by E. R. deOng and C. L. Roadhouse. Mr. Simmons states that "until more extensive tests are made to prove otherwise, in regard to each variety of cheese, cyanide fumigation should not be recommended." But he fails to recognize the fact that the bulletin referred to dealt with only one type of cheese, cheddar, and that in his own data which he cites, this variety absorbed but "a mere trace" of hydrocyanic acid gas.

The experiments performed by deOng and Roadhouse were on this one type of cheese only, as reported in the bulletin: tests on other varieties were purposely omitted as they are of little commercial importance in California.

Toxicity tests made by deOng and Roadhouse on white mice by allowing them to eat freely of the freshly fumigated cheese showed no ill effects whatever. In later tests the experimentors themselves ate from one to three ounces of the fumigated cheese, both that enclosed by the rind and freshly cut surfaces within one hour after removal from the fumigating room but without any ill effect.

Later experiments by the Federal Insecticide and Fungicide Laboratory as reported in United States Department Bulletin 1307, pages 6 and 7, show that American cheese when first removed from the fumigatorium might have as high as 110 parts per million of hydrocyanic acid gas but this drops to 55 p.p.m. in one day and 22 p.p.m. in seven days. The authors state that "the hard rind of an American Swiss cheese was enough, however, to prevent the gas from penetrating. None was found even close under the rind, although some was present in the rind." The authors failed to discuss their findings from the standpoint of toxicity and made no tests from this standpoint.

Hence, the authors of California Bulletin 343 conclude both from the data contributed by Perez Simmons and E. L. Griffin et al., as well as their own findings that their recommendations for the use of hydrocyanic acid gas as a fumigant for cheddar cheese should stand as given.

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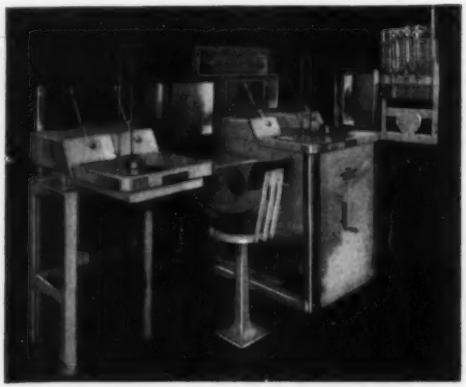

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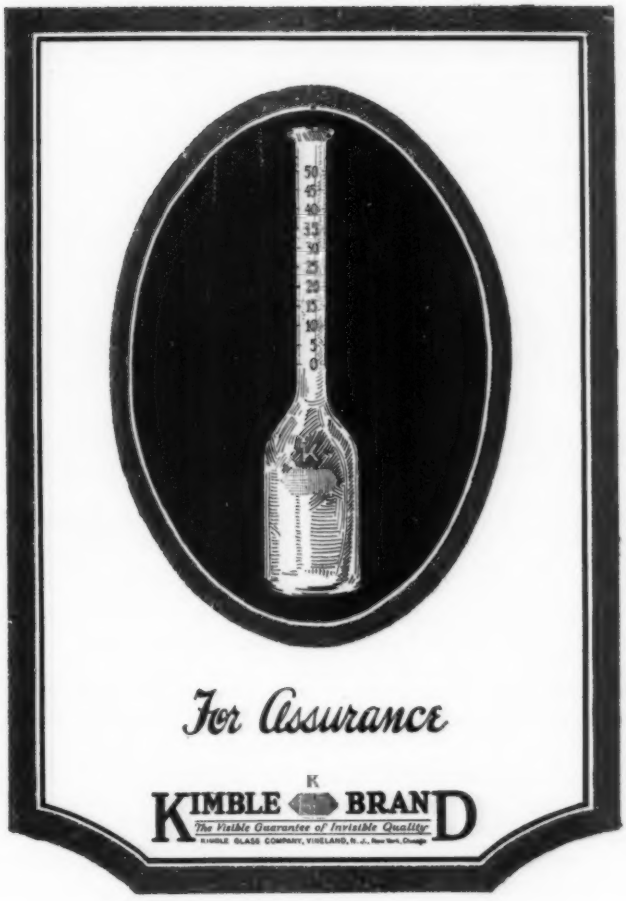
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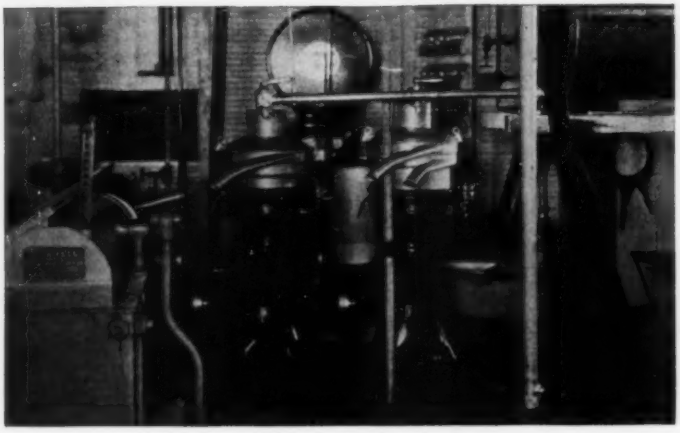

Greater Separator Durability

Reason No. 3 why a new De Laval will make you larger profits

With the right kind of care a De Laval Separator practically never wears out. Hundreds of machines installed ten, fifteen and even twenty-five years ago are still giving good service even though in many cases it would be advisable to trade them in on an improved De Laval.

De Laval Separators have always been built to last, but each year has brought some improvement to make them more durable. Several important improvements have recently been made—all of them adding to De Laval durability and dependability.

With the improvements which add to the durability of the De Laval Separator, changes have also been made which greatly simplify its construction and operation. The new De Laval requires fewer adjustments; it has fewer parts and the parts are placed and fitted so that adjustments or replacements are more easily made when necessary.

Sooner or later you will use a

De Laval

The De Laval Separator Company

NEW YORK CHICAGO SAN FRANCISCO

DE LAVAL DE LAVAL

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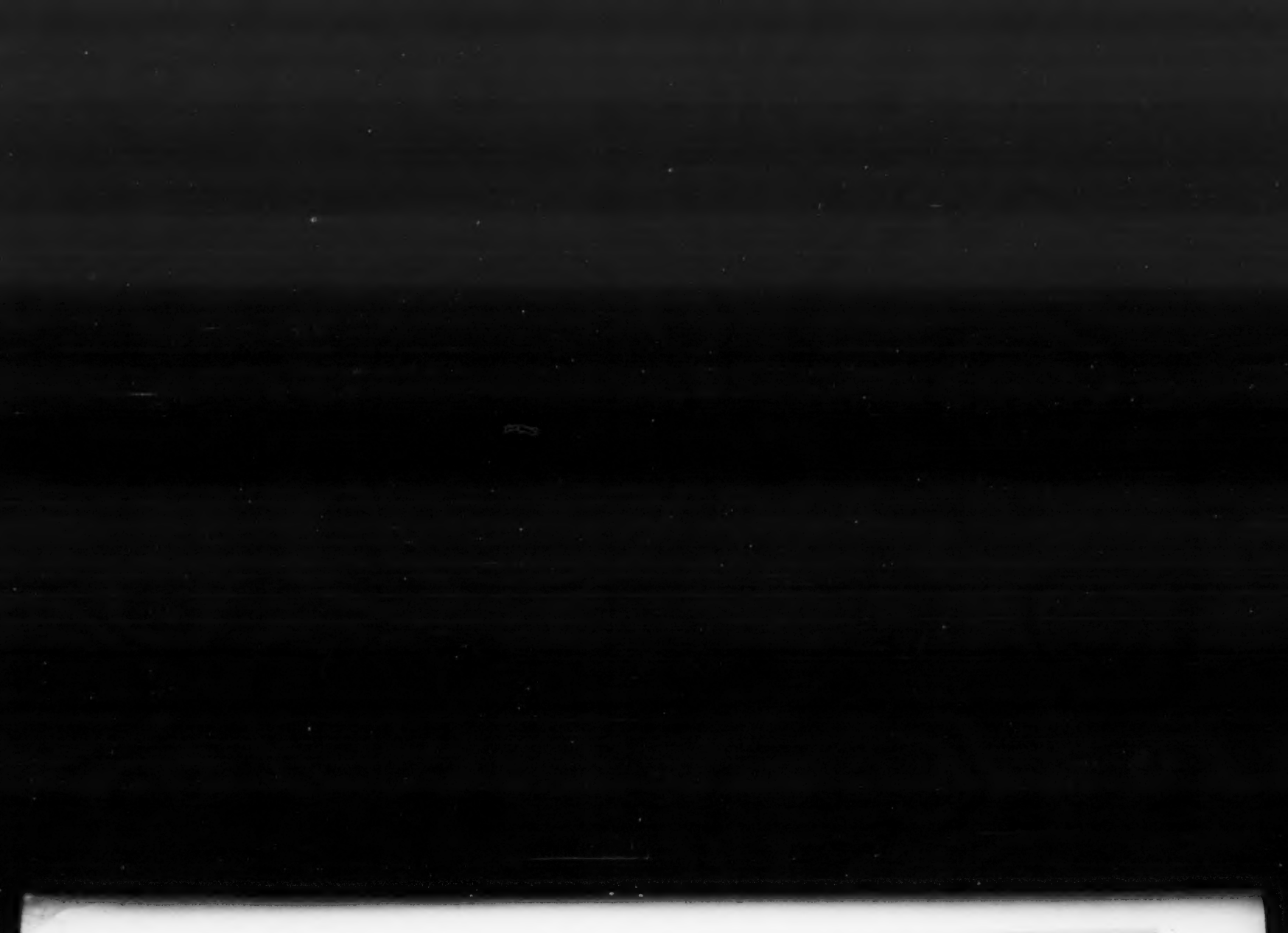

Wagner's Precision Milk and Cream Test Bottles with **Indestructible Black Graduation**, guaranteed not to decolorize; eye strain is practically eliminated, thereby insuring accuracy, efficiency, and contented operators; the easy reading gives a sense of secureness to the operator, it fortifies him against unjust claims; it makes retesting and rereading unnecessary; saving time and wages.

Some of the testimonials received, read:

"The Wagner Test Bottles with Indestructible Black Graduation are worth their weight in gold."

Order from us or your jobber.

The Wagner Glass Works
ESTABLISHED 1887
Manufacturers and Inventors of Glassware and Apparatus Pertaining to the testing of Milk and its products.
695-697 E. 132nd St.
New York, N. Y.
U. S. A.

The Aspergilli

By CHARLES THOM
and MARGARET B. CHURCH
of the Microbiological Laboratory of the Bureau of Chemistry, Department of Agriculture

One of every four or five molds encountered in handling general problems is an Aspergillus. The literature and references are scattered in some 300 places in nearly a dozen different languages. The present research has sought and found the original descriptions of all but three species, where secondary references had to be taken.

Complete key to the identification of the species included. Abbreviated key given for ready practical application.

Price $5.00

The Williams & Wilkins Co.
Publishers of Scientific Books and Periodicals
Baltimore, U. S. A.

Christensen's

Milk Grading Apparatus
SCIENTIFIC PRINCIPLES

Catalase Apparatus
PERFECT CONSTRUCTION

Propagating Apparatus
PRACTICAL OPERATION

Equipped with different accessories, *one* apparatus is used for all *three* purposes.

"The Milk Grader"

for determining the *bacteria in milk* and cream, especially mixed *herd milk*, by **The Reduction and Fermentation Test.** For 28, 60 and 200 25-cc. samples, or for 48, 90 and 300 10-cc. samples, as recommended by the American Public Health Association. Gas lamps, alcohol lamps or *electric control* which has proved of special value during two years. *Health Departments*, *Dairy Schools* and *Experts*, leading *Dairy Concerns* have adopted and endorsed "The Milk Grader."

"The Catalase Tester"

for detecting *blood* and *pus in milk*, due to abnormal milk secretion, caused by physiological conditions and *diseases* of the cow. For testing milk from *individual cows*, especially when marketed as **Certified Milk** or used raw for Cheesemaking, etc. Apparatus for 14 and 30 samples (20 cc.).

"The Culture Propagator"

for *propagation of cultures* and preparation of *starters* in enameled cans at any temperature required, whether *Lactic*, *Bulgarian* or *Acidophilus Culture*. A practical and reliable **Sterilizing Incubator** with electric control. The smaller apparatus for 3 and 6 pints mother starter, consist of "The Milk Grader" with a few accessories. Large apparatus for two mother starters and 8 gals. of starter, fitted for direct steam, water and brine connection.

Circulars on Request

OHIO FOOD & DAIRY LABORATORY

VALDEMAR CHRISTENSEN
Chemist and Micro-Biologist
145 East Fourth Street
CINCINNATI, OHIO

THE STANDARD FOR NEARLY FORTY YEARS

TRADE MARK

Paterson Vegetable Parchment

"Nearest to Perfection"

Nearly forty years ago we were making Vegetable Parchment. One does not specialize for nearly forty years without coming very close to Perfection.

The Paterson Parchment Paper Co.

Passaic, New Jersey

DIFCO

Standardized Reagents for Nutrient Agar

BACTO-BEEF EXTRACT

A uniform extract of lean beef prepared for use in culture media. It forms a sparkling clear aqueous solution of pH 6.5±.

BACTO-PEPTONE

A product of exceptional uniformity having a reaction of pH 7.0±. Most desirable for the preparation of standard nutrient Agar.

BACTO-AGAR

A purified agar prepared for use in bacteriological culture media.

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS

In the research and development of Bacto-Peptone and Dehydrated Culture Media

Digestive Ferments Company

DETROIT, MICHIGAN, U. S. A.